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Velvet antler removal from red deer

A thesis presented in partial fulfilment of the degree of

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For my family

My fiancé,
Paul Chambers
and my wonderful children,
Malina, Niko, Jessica, Robert, William and Thomas.

Short Abstract

Antlers are unique structures specific to deer that are cast and regrown annually. The harvest of velvet antler is a surgical procedure requiring analgesia and haemostasis. Analgesia is provided by lignocaine ring blocks around the base of the antler in adult stags or compression analgesia with a rubber (*NaturO*TM) ring in yearlings. Stags are often sedated with xylazine for velvet removal. This thesis covers four topics requiring refinement or further understanding: variability in local analgesia using lignocaine; the efficacy or noxiousness of *NaturO*TM rings; lignocaine residues in antlers; and post- xylazine stag death.

In a video observational study of antler harvest on commercial velvet farms, successful analgesia was characterised by fewer “gaps” in the ring block, greater volume of lignocaine per antler, greater time taken to inject lignocaine, greater number of injections, lower stag age, and other stag factors. There was significant confounding in the data and multiple variables are likely to be important.

In a series of studies investigating the use of *NaturO*TM rings, the rings prevented behavioural responses and partially prevented EEG responses to antler removal. On application of the *NaturO*TM ring, some animals demonstrated behavioural and EEG responses indicative of noxious sensation. *NaturO*TM rings provide analgesia during antler removal and may cause discomfort on application.

It is believed that lignocaine reaches the antler via the blood stream, resulting in tissue residues. Blood flow to the antler was investigated using acrylic models, fluorescent microspheres and cine-angiography. Different tourniquet types were tested to determine whether they could prevent lignocaine residues. The arterial supply to the antler is elaborate and tourniquets minimised arterial flow at tensions less than 2kg but did not prevent lignocaine residues.

Occasionally, stags die within 48 hours of sedation with xylazine. In an epizootological survey, this was not related to drug or environmental factors. Reactions involved either acute respiratory distress or delayed death with severe pulmonary oedema. During observations of yearlings sedated with xylazine most demonstrated hypoxaemia that could be reversed by oxygen supplementation or yohimbine administration.

This work provides indicators of how best practice in velvet antler removal may be improved.

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Ethics Approval

The experiments contained in this thesis have received approval from the Massey University Human Ethics Committee and the Massey University Animal Ethics Committee.

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List of abbreviations

		Chapter
ACVM	Agricultural Compounds and Veterinary Medicines	1, 5
AP	Antlerogenic Periosteum	1
AWAC	Animal Welfare Advisory Committee	1
BW	bodyweight	1
C	Close-up video recordings	2
cAMP	Cyclic adenosine monophosphate	1
CI	Confidence interval (statistics)	2
CNS	Central Nervous System	1
coeff	coefficient	2
CSF	Cerebrospinal fluid	1
DINZ	Deer Industry New Zealand	1
DMA	di-methyl-aniline, also referred to as 2,6-DMA, DMHA or 2,6-xylydine	1,4
DRU	Massey University Deer Research Unit	3,4,5
EA	Electroanalgesia	1
EEG	Electroencephalography, electroencephalogram	1,3
ESR	Environmental Science and Research (Laboratory)	1
ET	End Tidal (as in ET halothane concentration)	3
F50	Median Frequency (of a power-frequency EEG spectrum)	3
F95	95% Spectral Edge Frequency (of a power-frequency EEG spectrum)	3
faf	Focal Analysis File	3
fl	Flinch: Small sharp movement	2
fmf	Focal Master File	3
FMRC	Fluorescent Microsphere Resource Centre	4
GCMS	Gas Chromatography Mass Spectrometry	1,4
gdf	Global definition file	3
GLP	Good Laboratory Practice	4
HA	Head Arch: Movement of the head and poll so that the nose moves backwards and the poll moves upwards	2
HCO ₃	Bicarbonate (Blood gas analysis)	5
HD	Head down: Downwards displacement of the head	2
HPLC	High Performance Liquid Chromatography	4
HR	Head rotate	2
IARC	International Agency for Research on Cancer	4
IJEACCM	Interim Joint Expert Advisory Committee on Complementary Medicines	1
IV	Intravenous	1
IVRA	Intravenous regional anaesthesia	1
LC-MS	Liquid Chromatography-Mass Spectrometry	1,4

LPS	Lipopolysaccharide	5
MAF	Ministry of Agriculture and Forestry, New Zealand Government.	1
MEGX	Monoethylglycinexylidide	1
min	minutes	2
mL	millilitres	1,2,3,4,5
mm	millimetres	2
mmHg	millimetres of mercury - a unit of pressure, e.g. blood pressure	5
MPL	Maximum Permissible Level (synonyms MRL Maximum Residue Level, Maximum Residual Level, MPRL Maximum Permissible Residue Level)	4
MRI	Magnetic Resonance Image	1
NAWAC	National Animal Welfare Advisory Committee	1
NDLR	Negative diagnostic likelihood ratio	2
NPV	Negative predictive value	2
NSAID	Non-steroidal anti-inflammatory drug	1
NumVelv-Year	Number of stags velveted per year : The average number of stags that an operator velvets per year	2
NVSB	National Velveting Standards Body	1
NZDFA	New Zealand Deer Farmers Association	1
NZFSA	New Zealand Food Safety Authority	1,4
NZVA	New Zealand Veterinary Association	1
OR	Odds Ratio (statistics)	2
p	probability, statistics (as in p-value)	2,3,4,5
PaCO ₂	Arterial carbon dioxide pressure	5
PaO ₂	Arterial oxygen pressure	5
PDLR	Positive diagnostic likelihood ratio	2
pH	pH. A measure of acidity/alkalinity	5
PIM	Pulmonary Intravascular Macrophage	1,5
PPV	Positive predictive value	2
RNZSPCA	Royal New Zealand Society for the Prevention of Cruelty to Animals	1
TCM	Traditional Chinese Medicine	1
TNF	Tumour Necrosis Factor (as in TNF-alpha)	5
TP	Total Power	3
SaO ₂	Arterial haemoglobin oxygen saturation calculated from blood gas analysis	5
SD	Standard deviation (statistics)	2
sec	seconds	2
sl	slight: A small movement of the head	2
SpO ₂	Haemoglobin oxygen saturation (%)	5
stdev	standard deviation (statistics)	2
TCO ₂	Total carbon dioxide (arterial blood gas analysis)	5
TNF	Tumour Necrosis Factor	5

USFDA	United States Food and Drug Association	1
vax	Vaccination. As in vaccination gun	2
Vol	Volume, usually in millitres	2
vs	versus	
W	Wide angle video recordings	2
YrsVelv	Year velveting - The number of year the operator has been personally velveting stags	2
ZT	Zygomaticotemporal. Injection site for lignocaine for antler analgesia, below the zygomatic arch.	2

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Long Abstract

Antlers are unique structures specific to male deer that are grown and cast on an annual cycle and are unlike the horns of other ungulate species. During the growth phase, velvet antlers have a well developed neurological and vascular supply and are sensitive to pain. Antlers are usually removed from deer, for commercial and management reasons, during the velvet growth stage, when they are sensitive to pain and therefore analgesia must be provided. The removal of growing velvet antlers is a controlled surgical procedure that can only be carried out by a veterinarian or a farmer that has been certified by a veterinarian. There is considerable legislation surrounding the removal of antlers and the New Zealand Deer Industry has a desire to continue its right to harvest antlers and wishes to ensure that methods used are safe, effective and humane. The experiments in this thesis examine some areas of velvet antler removal that may be refined in order to improve the quality assurance of the procedure. These include analgesia with lignocaine and compression bands, drug residues and sedation.

Chapter one of this thesis provides background information on the anatomy and commercial harvest of antlers and introduces and discusses concepts and methods relevant to the experimental chapters (2 to 5) that follow. The general outline is as follows:

- Method for applying local anaesthetic ring blocks and determining a pain response
Introduced in Chapter 1.2, experiment in Chapter 2.
- Use of compression with the *NaturO*TM ring for analgesia in yearling stags
Introduced in Chapter 1.3, experiments in Chapter 3
- Lignocaine residues and the development of a tourniquet to prevent residues
Introduced in Chapter 1.4, experiments in Chapter 4
- Post xylazine stag death and the effects of xylazine on blood oxygen levels
Introduced in Chapter 1.5, experiments in Chapter 5

Lignocaine ring blocks

Under current practice in New Zealand, analgesia is provided by subcutaneous lignocaine injections in adult stags or *NaturO*TM ring compression bands in yearling stags. Experiments have shown that lignocaine injected in a high dose ring block around the base of the antler provides superior analgesia compared to blocking nerve trunks more proximally. Limited data from field observations of farmers applying lignocaine and removing velvet suggests that the achievement of analgesia in the field may be variable, with some operators achieving better results than others. There is a need to describe the best practice for applying lignocaine ring blocks to maximise the chances of analgesia for all stags on commercial farms. In order to do this a method for determining whether analgesia has been achieved needed to be developed. Previous methods have used a behaviour grading scale based on the amount of the stags body involved in the movement. This method may have limited sensitivity and/or specificity and there was a need to better define a

withdrawal response caused by pain sensation from antler that were cut with insufficient analgesia. Methods for assessing a pain response in animals are reviewed in Chapter 1.2, while Chapter 2 consists of an observational study of farmers and veterinarians harvesting velvet antler in the field. A detailed behaviour analysis was done to develop the criteria for determining a withdrawal response to antler removal. Using this behaviour scoring system, operators were ranked according to the proportion of stags that showed a withdrawal response during antler cutting. Variables associated with the farm, operator, animal and injection method were analysed to determine which had a significant effect on the likelihood of a withdrawal response during antler removal. “Flinch” had a higher sensitivity for a withdrawal response compared to previously described movement of the head, neck or body. “Flinch”, “Head arch”, “Head down” and “Head rotate” had greater specificity for a withdrawal response compared to previously described movements and a combination of these specific head-related movements is likely to be a more accurate predictor of failed analgesia than previously described behaviours. The overall withdrawal response during commercial antler harvest was 35% and ranged from 0 to 76% between operators. The most important factor influencing the likelihood of a withdrawal response was the presence or absence of visible “gaps” in the ring block, whereby injection sites did not overlap. Other variables that were important included the number of injections, total volume injected and injection time. Factors relating to the stag including stag age, sedation and stage of antler growth also appeared to have an effect on behaviour during antler removal although there was a high level of confounding in the data. An interaction model was drawn and recommendations made for best practice injection method.

NaturO™ ring

Compression analgesia by the use of *NaturO™* rings is reviewed in Chapter 1.3 and four experiments relating to this topic are presented in Chapter 3. Compression analgesia has been investigated in deer and is currently legal in New Zealand for the removal of velvet from one-year-old stags using *NaturO™* rings. This method of analgesia provides a solution to problems concerning drug residues in velvet antler and transport of yearling deer following velvet removal. Compression causes displacement of myelin sheaths and hypoxia of nerves, causing them to cease functioning within a time period that is related to the level of pressure applied by the compression device. There are limited studies on the efficacy of compression for preventing a pain response to velvet antler removal or on whether compression is itself noxious to the animal. Chapter 3 of this thesis details four experiments using electroencephalography (EEG) and behaviour to help determine whether *NaturO™* rings prevent a response to antler removal and also to determine whether *NaturO™* rings cause a pain response when they are applied to the animal. When antler were cut from anaesthetised stags that had a *NaturO™* ring applied to the pedicle, the average EEG response was somewhere between that of stags that had been treated with lignocaine ring blocks and those that had no treatment. There was no difference in the behaviour of yearling stags during velvet antler removal that had been treated with either a *NaturO™* ring or lignocaine ring block prior to antler removal. When comparing responses to application of *NaturO™* rings and lignocaine ring blocks, there were significant differences in the EEG pattern following application of the different treatments and this was mostly due to changes in the lignocaine group. Overall, there was little effect on the mean EEG parameters in the *NaturO™* ring treated stags but on an individual animal basis, seven out of 10 stags treated with *NaturO™* rings showed some significant changes consistent with noxious somatic stimulus. Stags that were videoed for one hour following

application of one of a combination of *NaturO*TM rings, lignocaine or no treatment did not show differences in the majority of normal behaviours. Stags that were treated with *NaturO*TM rings had a higher frequency of some specific head related movements such as head rotating, turning head in circles, arching the head up or down and head pressing. Overall, the *NaturO*TM ring appears to offer analgesia during antler removal but may be perceived as noxious on application in some spikers but not others.

Lignocaine residues

Lignocaine use and safety is reviewed in Chapter 1.4 and the experiments relating to lignocaine residues are presented in Chapter 4. While compression analgesia ensures no residues are present in the antler, it is currently not an accepted form of analgesia for adult stags and lignocaine hydrochloride is used for analgesia in adult stags. One metabolic product of lignocaine, 2,6 dimethyl-aniline is classified as a potential carcinogen to humans after studies on the carcinogenic effects on rats. The maximum permissible level for lignocaine in velvet antler is set at 1mg/kg. This level is frequently exceeded in antler tissue so a method of preventing lignocaine residues was sought. It was believed that lignocaine reached the velvet antler via the blood stream and that a tourniquet placed prior to lignocaine injection would prevent lignocaine residues. Fluorescent microspheres, cineangiography and direct observations were used to determine the tourniquet tension required to minimise antler blood flow. In most cases tourniquets applied at a tension of 2kg or less prevented antler blood flow. Efforts to develop a consistent tourniquet for use in the field were unsuccessful at preventing lignocaine residues in the antler suggesting that either tourniquets are unable to fully prevent leakage of blood and lignocaine into the antler or that lignocaine is reaching the antler by another route.

Xylazine reactions

Stags often require sedation for velveting to ensure the safety of both the stag and the operator. The most commonly used drug for this is xylazine. Xylazine is prescribed and distributed to farmers that are licenced to harvest velvet from their own animals. Stags occasionally die up to 48 hours after administration of xylazine and this has generally been described as an anaphylactic or delayed anaphylactic reaction. Deaths are sporadic and variable from one year to another. Little is known about the factors that cause stag death following sedation with xylazine or the exact mechanism of the pathology. Chapter 5 of this thesis includes two main areas of research, a survey study of stag deaths and a series of experimental studies and observations of the hypoxaemic effects of xylazine in young deer. Survey data was limited and unable to identify specific risk factors although a number of potential causes were able to be excluded. Deaths were not related to any environmental factors including weather, temperature, time of day, facilities and method. The formulation and dose of drug administered was not a contributing factor, nor was the administration of the reversal agent, yohimbine. Deaths were sporadic in location and time, with some farms experiencing multiple deaths and others never experiencing a death. A limited number of postmortem samples were sent to Massey University for histopathology. The majority of these revealed pulmonary oedema with or without pulmonary haemorrhage. Yearling deer sedated with xylazine demonstrated marked hypoxaemia measured as reduced SpO₂ and PaO₂ levels. This

was reversed after administration of endotracheal oxygen or reversal with yohimbine. It is hypothesised that xylazine causes hypoxaemia and pulmonary oedema in nearly all deer and that in most cases this is transient but a population of deer experience severe hypoxaemia from which they do not recover. Supplemental oxygen is recommended for all deer given xylazine or xylazine combinations and susceptible stags should be identified and alternative methods of restraint used.

Chapter 6 identifies some potential future research options that may help to further refine the methods used for velvet removal making the procedure safer and more humane for the animals and more predictable for the assurance of the general public. Potential options include the development of an automated needle-free drug delivery device, the use of a different local anaesthetic agent that does not produce carcinogenic metabolites and the use of peripheral alpha-2 antagonist agents to mitigate the hypoxaemic effects of xylazine in deer. These methods could also be extended to use in other ruminant species.

Chapter 1

General introduction

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Note to reader

Due to the large volume of information in this thesis and multiple experiments in each experimental chapter (2 to 5) an effort has been made to shorten the experimental chapters and to avoid repetition of introductory notes and literature review. This has been done by providing a detailed introduction and literature review in Chapter 1, covering the principles and methods used. Where possible, the introduction section relating to each chapter is numbered accordingly, such that Chapter 1.2 largely relates to the experiment in Chapter 2; Chapter 1.3 relates to the experiments in Chapter 3, etc. When reading the experimental chapters, only a brief introduction will be found with cross reference back to the relevant section of Chapter 1.

Introduction to Chapter 1

Velvet antler removal is a controversial subject with countries adopting different philosophies and legal status. e.g. the procedure is restricted to emergency situations only in the UK (Veterinary Surgeons Act (England) 1966). In New Zealand, velvet antler removal is allowed under strict regulations. The New Zealand deer industry wishes to maintain “Freedom to operate” and the right to harvest velvet antler for commercial purposes without imposing trade barriers. Farming practices and particularly painful husbandry procedures are under increasing scrutiny from consumers and retailer who are demanding food that is safe to eat, nutritious, free of drug residues, and has not caused harm to the environment or the animals in its manufacture (Hoffman, 2000; Verbeke and Viaene, 2000). One product (“Antler Volver Full Spectrum”, Planetary Herbals, Soquel, CA) is marketed as “Carefully harvested in accordance with the National Velvet Standards Body in New Zealand”. In order to maintain the right to harvest velvet antler, the deer industry must satisfy its customers that the best, safest and most humane methods are being used and that ongoing research is continually improving these methods. This thesis addresses four aspects of the current method for harvesting velvet antler that require improvement, review or increased understanding. These are: the application method of local anaesthetic; the use of *NaturO*TM rings; the presence and prevention of drug residues in antler; and the effects of the sedative drug, xylazine on deer. These topics have been researched at the request of, and with funding from, the deer industry.

This introductory section begins by describing the commercial harvest of velvet antler and the legislation surrounding velvet antler removal. The anatomy of velvet antler is described with particular reference to the neurological and vascular supply to the velvet antlers in order to understand the effects of placement of lignocaine and tourniquets and the potential occurrence of lignocaine residues in antler. A large part of the work in this thesis is on determining whether lignocaine and compression methods provide effective analgesia for velvet antler removal. These and other methods of analgesia are reviewed in detail. The methods for detecting a pain response or lack thereof are not well understood in deer and some of the methods developed in this thesis involved the refinement of behavioural analysis for determining a pain response. Methods of assessing pain and analgesia are reviewed here with specific reference to deer and hopefully, the methods adopted in the experiments of this thesis are justified. The metabolism and potential carcinogenic effects of lignocaine are reviewed with respect to lignocaine residue in velvet antler and finally, the use and effects of xylazine are reviewed so that the methods used to further our understanding of post xylazine reactions could be developed.

1.1 Velvet antler production in New Zealand

1.1.1 Velvet antlers as a commercial product

Deer are farmed in New Zealand on pastoral land for the production of export venison, velvet antler and by-products. Velvet antler makes up approximately ten percent of the export earnings from deer and for the 2010/2011 season totalled 434 tonnes at a value of around 26 million dollars (DINZ annual report, 2011). Ninety four percent of this is sold to the East Asian regions of Korea (43%), Hong Kong (10%) and Mainland China (41%). A small amount (2%) is exported to the USA.

Velvet antler refers to the entire growing antler above the bony pedicle from which the antler is cast and re-grows every year. It includes the skin, hair and underlying cartilaginous material that gives antlers their shape. The entire antler is frozen or dried and processed and different parts of the antler are incorporated into different Traditional Chinese Medicines (TCM) and used for the treatment of ailments and the enhancement of health. A small amount is marketed as a health supplement for athletes and pets. The pharmaceutical properties of velvet antler will not be covered here but have been reviewed (Haines and Suttie, 2008).

During the past 40 years, since deer were first legally farmed, there has been genetic selection for antler weight and conformation resulting in higher yields and greater economic returns. A good stag can grow more than 5 kilograms of velvet antler each year. At an average price of \$80 per kilogram, this represents \$400/stag/year income. This would be equivalent to approximately \$150 per stock unit and thus competes well with other farming enterprises. The price of velvet antler is neither stable nor predictable and has fluctuated from as high as \$247/kg in 1990 to a low of \$40/kg in 1999 for A grade velvet. Nevertheless, velvet remains a viable commercial enterprise which is partly due to the low maintenance costs and minimal health problems of velvet producing stags.

Most deer farmers in New Zealand will remove antlers from male deer at some stage and the majority of these are removed during the velvet stage of growth. Even in venison only production systems, breeding stags will usually have the antlers removed to ensure the safety of the deer and the farm staff. In addition to breeding stags, young male deer will grow antlers in their first year of life and these may have to be removed before they are transported to venison processing plants.

1.1.2 Legislation concerning the removal of velvet antlers

Velvet antlers are removed from live animal and are considered to be “harvested”, as the new antler will regrow the following year. This can be compared to the annual harvest of wool from sheep. Harvesting of the antlers in one year will not affect the growth of velvet in subsequent years. Harvesting of velvet is not believed to affect the health or physiology of the animal, nor is it considered detrimental to the social or behavioural needs of the deer. The primary differences

from harvesting wool is that the antlers are used as a food product and are harvested before they have lost their innervation and blood supply. It must also be removed and handled in a way that ensures food safety for the consumer and is not painful to the animal.

Velvet removal is a “controlled surgical procedure”

Removal of antlers can cause haemorrhage and pain (Matthews et al, 1992; Pollard et al, 1992a, b). Under New Zealand law, the removal of velvet is classified as a controlled surgical procedure requiring appropriate surgical technique, haemostasis and pain relief.

According to the Animal Welfare Act 1999, section 18, subsection (1) “A controlled surgical procedure may be performed on an animal only by - a) a veterinarian; or b) a person who is acting under the direct supervision of a veterinarian and who is being taught veterinary science at undergraduate level; or c) a person who is both the owner of the animal and a person who has veterinary approval to perform a surgical procedure of that type on that species of animal; or d) a person who is both an employee of the owner of the animal and a person who has veterinary approval to perform a surgical procedure of that type on that species of animal”. and section 18, subsection (3) “A person, being the owner of an animal or an employee of an owner of an animal, has, for the purpose of subsection (1), veterinary approval to perform a controlled surgical procedure on an animal only if a veterinarian certifies in writing, before the procedure is performed on the animal, that the veterinarian is satisfied that the person has the relevant expertise, practical experience, drugs, equipment, and accommodation to perform the type of surgical procedure competently on that species of animal”.

Thus it is a breach of the Animal Welfare Act 1999 for any person other than a veterinarian to remove velvet antlers from deer of any age without written certification.

The veterinarian’s legal obligations

During any surgical procedure the animal must be provided with sufficient analgesia, in the form of general anaesthesia or local analgesia so that the animal does not experience any unnecessary pain or distress (Animal Welfare Act 1999). The actions of the veterinarian are accountable to the Veterinary Council which has a mandate under the Veterinarians Act 2005 to ensure that veterinarians are fit to practice. Veterinarians are advised to comply with the Code of Professional Conduct for veterinarians and failure to do so may result in removal of the right to practice or to prosecution.

Velvet antler removal is not included in the Animal Welfare (Painful Husbandry Procedures) Code of Welfare 2005 or in the Code of Welfare for Deer 2007 although the latter does cover the husbandry, management, yarding and restraint of velvet stags. A separate code of recommendations and minimum standards for the welfare of stags during the removal of antlers was written by the then Animal Welfare Advisory Committee (AWAC) in 1992 under the now repealed Animal Protections Act, 1960. This code is somewhat outdated and refers to government organisations that no longer exist and methods that have been superseded. The accepted procedure is detailed more appropriately in the National Velveting Standards Body Farmer Velvet Antler Removal

Manual published by DINZ (Anon, 2005). This manual forms the basis of the training required for the controlled surgical procedure of velvet antler removal under the National Velveting Standards Body's (NVSBS) contractual agreement between veterinarians and farmers and is an up to date resource. This is the main vehicle by which new research and knowledge concerning velvet antler removal methods will be adopted and disseminated to farmers and veterinarians.

There is a legal requirement to provide analgesia for velvet antler removal but the exact method of providing analgesia is not specified. Veterinarians may use any method that they deem suitable for the purpose while complying with the Agricultural Compounds and Veterinary Medicines (ACVM) Act 1997, the Medicines Act 1981 and the Veterinarians Act 2005 and following the Veterinarians Code of Professional Conduct. It is in the interest of the veterinarian to follow recommended procedures to avoid prosecution should the analgesia be deemed insufficient. Alternative methods should only be used if the veterinarian has strong evidence to support their efficacy.

Veterinarians must also comply with the requirements concerning the purchase, storage, administration and sale of medicines used and ensure there are no residues in product destined for human consumption. Under the Agricultural Compounds and Veterinary Medicines (ACVM) Act 1997, section 62, there is a Performance and Technical Standard relating to the purchase, use, (and storage in anticipation of use) of xylazine, yohimbine and lignocaine (Develvetting Restricted Veterinary Medicines) used to facilitate the removal of deer velvet antler intended for human consumption.

National Velveting Standards Body (NVSBS)

Following publication and distribution of the Code of Recommendations and Minimum Standards for the Welfare of Deer During the Removal of Antlers, it became evident that a national programme was required to ensure that this code was applied to all velvet removal on farms (Campbell, 1993). Members of the The New Zealand Veterinary Association (NZVA), New Zealand Deer Farmers Association (NZDFA), the then Game Industry Board (GIB), Royal New Zealand Society for the Prevention of Cruelty to Animals (RNZSPCA), and branches of the Ministry of Agriculture and Forestry (MAF), MAFPolicy and MAFQual came together to form a committee to oversee a national programme. This joint body was called the National Velveting Standards Body (NVSBS) and was formed on 28 April, 1993 and remains in place today. This body administers a programme whereby farmers may be trained by veterinarians to remove velvet from their own deer under the indirect supervision of a veterinarian and includes information and training concerning the dispensing, storage and use of veterinary medicines, lignocaine, xylazine and yohimbine. According to the Animal Welfare Act 2005, a veterinarian may train a farmer and certify that farmer as competent to perform the surgical procedure, however under the NVSBS scheme there are several benefits and all farmers and veterinarians involved with velvet removal should be registered with the NVSBS. The NVSBS scheme also provides assessing and auditing veterinarians so that the training programme can be double checked by an independent veterinarian and ensure quality control for all in the industry (campbell, 1994). This is particularly important should the practice come under the scrutiny of animal welfare investigations.

1.1.3 Form, function and growth cycle

Figure 1.1.1 shows the annual growth cycle of deer antlers and Figure 1.1.2 shows the form and internal structure of growing antlers. Antlers are structures grown exclusively by the males of most species of deer (Goss, 1983). Antlers are quite different from the horns of bovids in their growth and structure (Davis et al, 2011). Horns remain throughout the animal's life and are rarely shed. Horns consist of a single spiked bony core around which a sheath of keratin like material which is deposited from the base. Antlers are multi-tine structures that are the only live mammalian tissue to be cast and regrow each year (Price et al, 2005). Antlers are separated from a short extension of the frontal bone (pedicle) by an intermediary zone, being the point at which the antler separates from the skull and regrows each year, whereas the core of horns is contiguous with the frontal bone. The reason that deer evolved to grow annually deciduous antlers is not fully understood but it has intrigued man for many centuries. Aristotle argued that antlers were in fact a disadvantage to the animal, having no useful function and that the best defence of deer was flight rather than using the antlers for fighting. When deer are born there is no evidence of antler development. At about 8 months of age male deer start to develop their pedicles from special periosteum of the frontal bone called anterogenic periosteum (AP) (Li and Suttie, 1994). This AP can be transplanted onto other parts of the body and will produce antler growth under the influence of testosterone (Goss and Powel, 1985). Castration before this time prevents pedicle development. The pedicle is a bony extension of the frontal bone that remains throughout the stag's life, whereas the antler is cast and re-grown from the end of the pedicle each year. Thus there are two distinct phases in antler development. Firstly the establishment of the pedicle that occurs once in the animal's life and secondly the annual antler growth cycle.

Antler growth, casting and regeneration is regulated by daylength and circulating hormonal levels (West and Nordan, 1976). Each year, the hard antlers are cast abruptly in the spring (Goss et al, 1992) when stag testosterone levels are at an annual low. The antlers immediately start to regrow after casting and are soft, vascularised, innervated and sensitive structures during the growing phase which lasts about four months. Antler growth occurs from the tip of the antler from pre-chondral cells that develop through a process of cartilage generation and ossification (Price et al, 1996) with the most calcified region closest to the skull (Figure 1.1.2). This process is under the influence of several different cellular mediators (Price et al, 2005). Elongation can occur at up to 20 millimetres per day which makes antlers probably the fastest growing tissues of land mammals. Blood vessels and nerves must elongate at the same rate as antler growth and branch with the forming antler tines. It has been postulated that the reason for a sensitive nerve supply is to allow the stag to protect his antlers from damage during the vulnerable growing stage (Goss, 1983). Low testosterone levels at this time also cause the stags to be docile and tolerate close company without aggression thus preventing damage to the growing antlers.

For humane velvet removal in velvet stags, a thorough understanding of the anatomy of the nerve and blood supply is required so that the best surgical technique can be used. Figures 1.1.3 and 1.1.4 show the blood and nerve supply to the antler respectively.

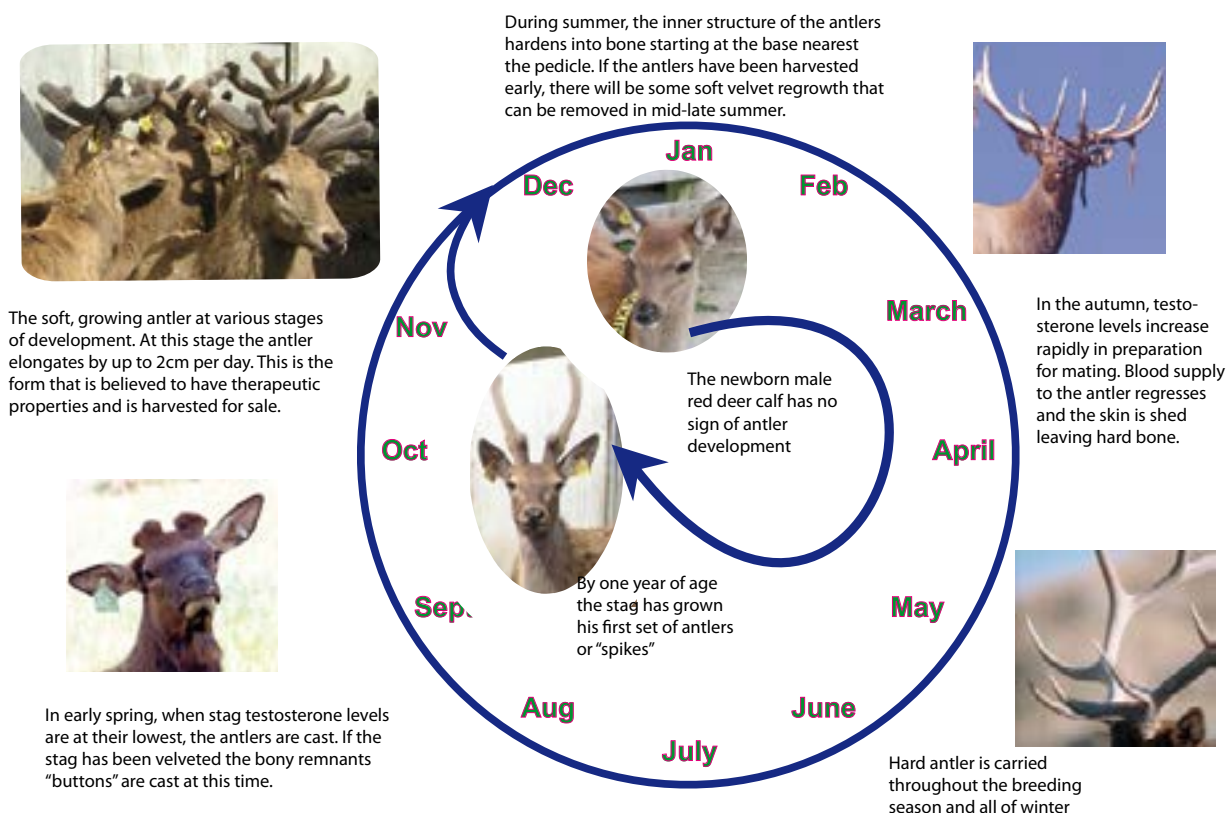


Figure 1.1.1 Annual antler growth cycle in seasonal red deer

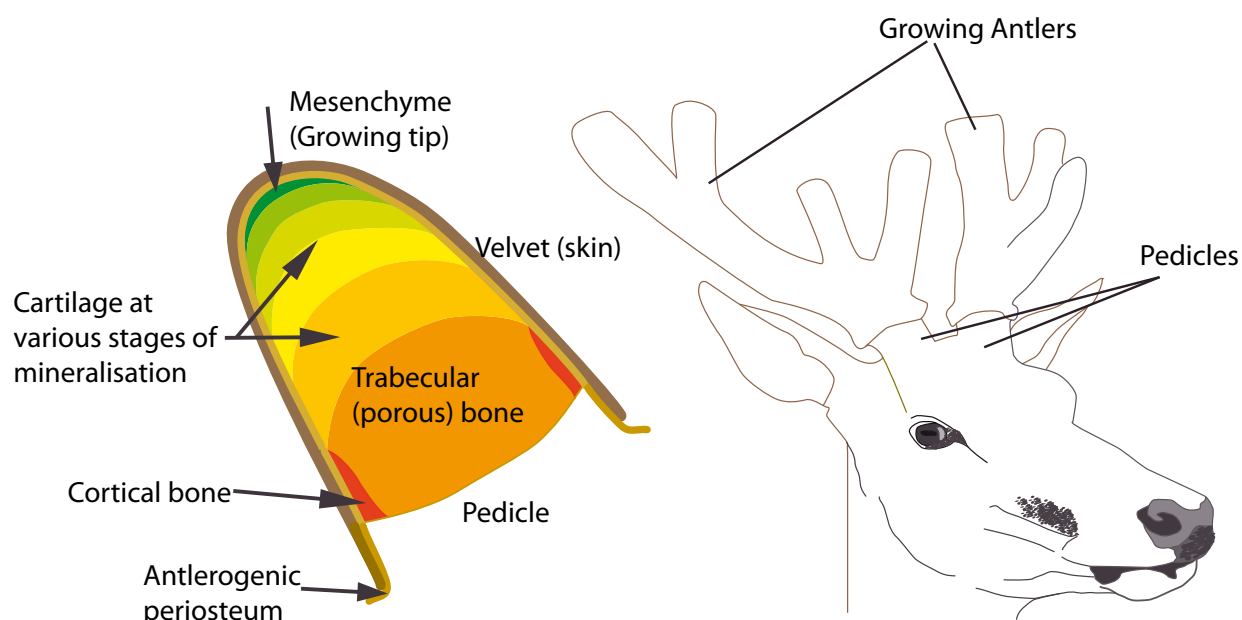


Figure 1.1.2 Schematic illustration of deer antler growth and mineralisation

Blood supply

Adams (1979) dissected 12 fresh or embalmed adult male red deer heads although it was not stated whether these were in active antler growth. In three of the heads, radiopaque medium and then latex was injected into the carotid artery and jugular vein to identify the arterial and venous branches. In these casts, it was observed that all of the blood supply to the antler was provided via the superficial temporal artery. This artery runs rostradorsally from the base of the ear and may be digitally compressed at this point to provide haemostasis if required. Above this level two main branches extend around the caudal and medial sides of the pedicle and about 9-12 further branches travel dorsally up the pedicle towards the antler. This is an unusual distribution of blood supply because the arterial supply is mainly peripheral. Adams observed that anastomoses were few and too small to be seen with the naked eye.

Adams (1979) described the venous drainage approximately following the arteries with several small veins draining the pedicle into two relatively larger vessels that travel around the lateral and medial aspects of the pedicle. These drain into the superficial temporal vein.

Suttie et al (1985) injected radio-opaque contrast medium into the carotid artery at various stages of antler development in young stags through a previously implanted prosthetic. These studies confirmed that the blood supply to the antler of yearling stags derives entirely from the lateral branch of the superficial temporal artery. In addition, these angiographs showed a prominent branch of the superficial temporal artery crossing from the pedicle to the antler itself by a contorted route following the contour of the coronet at the caudal aspect. A capillary bed was seen at the tip of the growing antler. Smaller arteries were also seen crossing the coronet rostrally and some appeared to terminate on the pedicle. These authors suggested that the anatomical structure observed supported the theory that the arterial supply to and upon the antler is peripheral and the venous drainage is mainly through the central core. It has not been identified or in fact suggested as to how the venous drainage ends up in the superficial temporal veins. This is of relevance when considering drug residues in antlers as some clinicians have suggested that drugs may enter the antler by backflow through a central venous sinus structure. Furthermore, when the antler is removed just above the coronet, blood is often observed oozing from the central region of the cut stump and this is not abated by tourniquet compression around the periphery of the pedicle. Removal of the tourniquet results in pulsatile arterial spurting from the periphery of the antler stump (personal observation).

Nerve supply

Adams (1979) described the nerve supply in embalmed dissected heads from red deer (*Cervus elaphus*) as coming mainly from the infratrochlear and zygomaticotemporal branches of the trigeminal nerves (cranial nerve V). The infratrochlear nerve emerges near the medial canthus of the eye and passes over the dorsal rim of the orbit. From here its branches pass through the orbicularis oculi muscle to supply the frontal regions of the head and branches pass caudomedially to innervate the rostral and medial aspects of the pedicle. The zygomaticotemporal nerve travels caudolaterally through the periorbita, emerging from the caudal margin of the zygomatic process of the frontal bone. This nerve then passes caudodorsally through the retro-orbital fat and beneath



Figure 1.1.3 Vascularisation of the antler pedicle showing the approximate distribution of the arterial supply (red) and venous drainage (blue) following descriptions by Adams (1979) and Suttie et al (1985).

the frontalis muscle. It then divides into several branches which disperse on the caudal and lateral aspects of the antler pedicle. Woodbury and Haigh (1996) dissected six wapiti and eight fallow stags heads and found the pattern of innervation from the zygomaticotemporal and infratrochlear branches of the trigeminal nerve to be very similar to the described by Adams in red deer.

Adams identified branches of the zygomatic branch of the auriculopalpebral nerve (a division of the facial nerve (cranial nerve VII)) that could be traced to the lateral surface of the pedicle. Woodbury and Haigh paid special attention to this nerve in their dissections and similarly found that a dorsal branch of this nerve travelled towards the antler in most cases but could not always be traced to the level of the pedicle.

The facial nerve is generally considered to carry efferent fibres and the sensory contribution of this nerve is unclear. The observation that an additional local anaesthetic block placed across the region between the ear and the antler often reduces a withdrawal response supports the proposition that the dorsal branch of the auriculopalpebral nerve may in fact carry mixed fibres, some of them including pain sensory axons (Wilson et al, 1999).

There has been no evidence to show that the cervical nerves contribute to the innervation of the

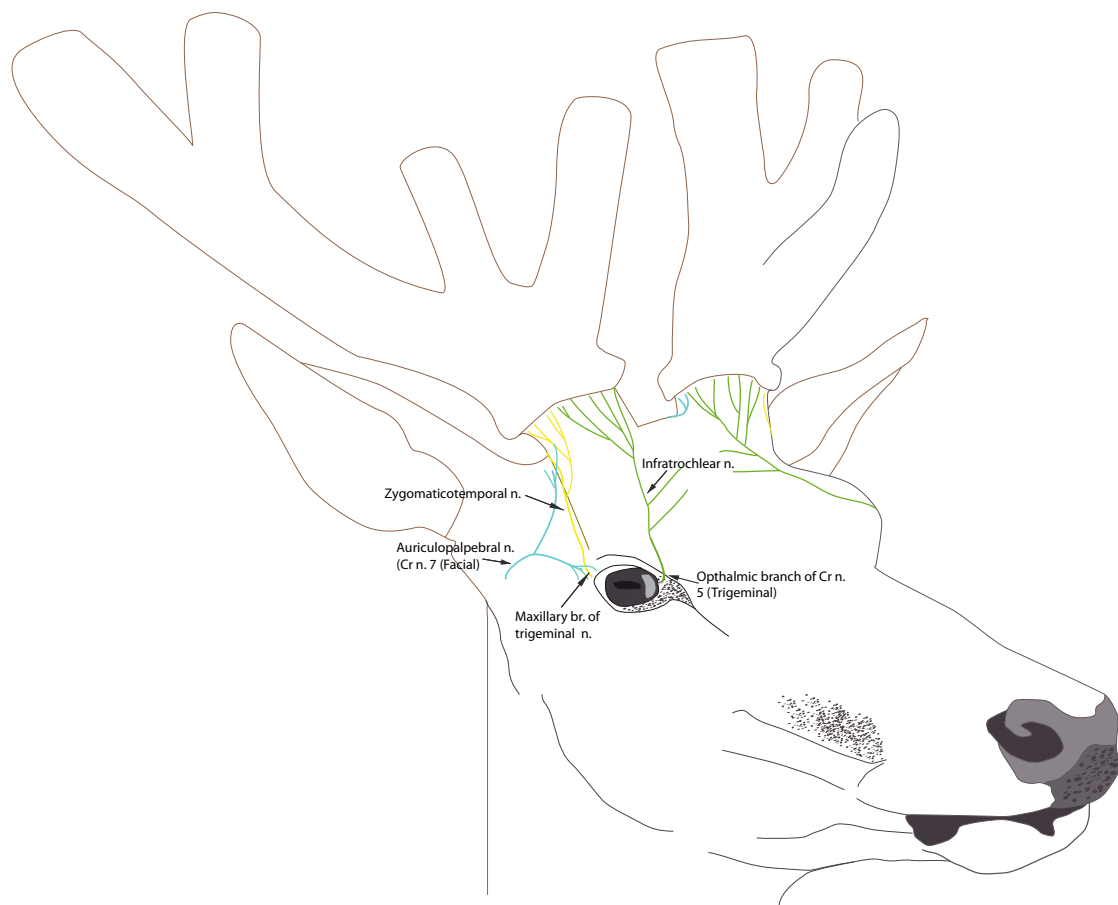


Figure 1.1.4 Innervation of the antler pedicle showing the approximate course of the zygomaticotemporal (yellow), infratrochlear (green) and auriculopalpebral (blue) nerves and their branches following descriptions by Adams (1979) and Woodbury and Haigh (1996).

antlers. Adams did not find any connections from the first two cervical nerves to the antler and could only trace these nerves to 4 cm from the pedicle. Similarly, Woodbury and Haigh (1996) found that in all deer branches of the second cervical nerve terminated at the base of the ear and did not reach the antler pedicle.

While branches of the infratrochlear, zygomaticotemporal and auriculopalpebral nerves described above can be traced to the antler pedicle this does not necessarily imply that the antler itself contains pain receptors to pressure, heat or cold. There are no experiments that trace the nerves through the junction between the pedicle and antler (coronet) to nerve endings in the antler itself. There have been experiments done identifying nerves within the antler. Wislocki and Singer (1946) examined histological sections of velvet antler and showed that nerve axons lie in bundles in the vascular layer beneath velvet skin. Vacek (1955) observed that myelinated and unmyelinated nerve fibrils were present and were most abundant in the hair follicles and intervening epidermis of antler velvet. Nerve fibrils were less abundant in the antler compared to normal skin. Vacek described nerve fibrils associated with arterial walls but Rayner and Ewen (1981) using fluorescence methods, showed that adrenergic nerve fibres were not present in arterial walls and concluded that antler arterioles do not undergo vasoconstriction. The structure of antler arterial walls was quite different to that of the pedicles and contained large cells that resembled mast cells. Rayner and

Ewen thus concluded that the antlers are probably not used as heat radiators for control of body temperature and that vasoconstriction probably does not cause vascular regression and antler skin necrosis when the velvet is shed in the autumn. Woodbury et al (unpublished) performed cross sections of antlers at the time of commercial velvet harvest and used a neurofilament polypeptide antibody to identify the location of nerve fibrils across the antler slices. No neurofilaments were present within the core of the antler and all sections that contained neurofilaments were close to the skin surface. Small and large fibres were differentiated and their relative distributions determined but no strong pattern was identified. It was concluded that the surface of the antler skin was well innervated all over but that these innervations were not associated with piloerection or vasoconstriction, suggesting that the innervation of the antler exists primarily for nociception and may be designed for the protection of the vascular and soft cartilagenous components of the antler.

1.2 Velvet antler pain: Mechanism, assessment and prevention

Experiments in Chapters 2 and 3 of this thesis use behaviour and electroencephalography to assess the efficacy of lignocaine and compression analgesia for velvet antler removal. In order to determine the efficacy of any analgesic, a reliable method for detecting a pain response, or lack thereof, must be used. This is difficult in any non-verbal person or animal that can not directly communicate their perception of pain. The following review describes the pain pathway during velvet removal and ways that this can be assessed for functionality. Methods that have been used to block the pathway and provide analgesia are also reviewed in this section.

1.2.1 Velvet antler pain mechanism

Figure 1.2.1 shows the predicted pathway of velvet antler pain initiation, processing and outcomes along with the sites at which the pathway can be blocked or inspected for function or cessation of function. The text and illustrations in red show the pain conduction, processing and outcome pathway. Those in blue show potential ways that the pathway can be blocked and the text and illustrations in green show potential ways of measuring activity in the pain pathway and the advantages and disadvantages of each method.

Current knowledge on pain mechanism has been thoroughly amalgamated by the International Association for the Study of Pain (Charlton, 2005). With respect to velvet antler, very little is known, but extrapolation from general principles leads to the following proposition: The pathway starts with cutting the nerves in the velvet antler and this initiates an action potential in the afferent nerve fibres. At some point along the pathway the signal is likely to undergo modification from other nerve cells or circulating neuromodulatory compounds (Melzack and Wall, 1965; Melzack, 1999). Afferent signals will be processed in various brain centres: perception of pain is probably generated in the frontal cortex. A response is generated to serve the purposes of stopping the damage, avoiding further damage and dealing with the damage that has been done and allow healing (Seksal, 2001). These may be most evident to the observer as withdrawal and avoidance behaviour and an attempt to escape or flee. The hypothalamic-pituitary-adrenal axis associated with stress is also likely to be activated in response to velvet antler pain. It is not known whether an emotional response to pain is experienced by deer.

Figure 1.2.1 (next page). Diagrammatic illustration of the predicted pain conducting, recognition and response pathway to noxious stimulation of the velvet antler (shown in red text and outlines). This begins with noxious stimulation of the antler (red and yellow star), followed by afferent nerve conduction, cerebral processing, efferent nerve conduction and outcome responses. Also shown are the points at which the function of the pathway can be assessed (green text and boxes). The advantages and disadvantages of each method of pain pathway function assessment are shown. The blue text and boxes show points at which the pain pathway can potentially be blocked.

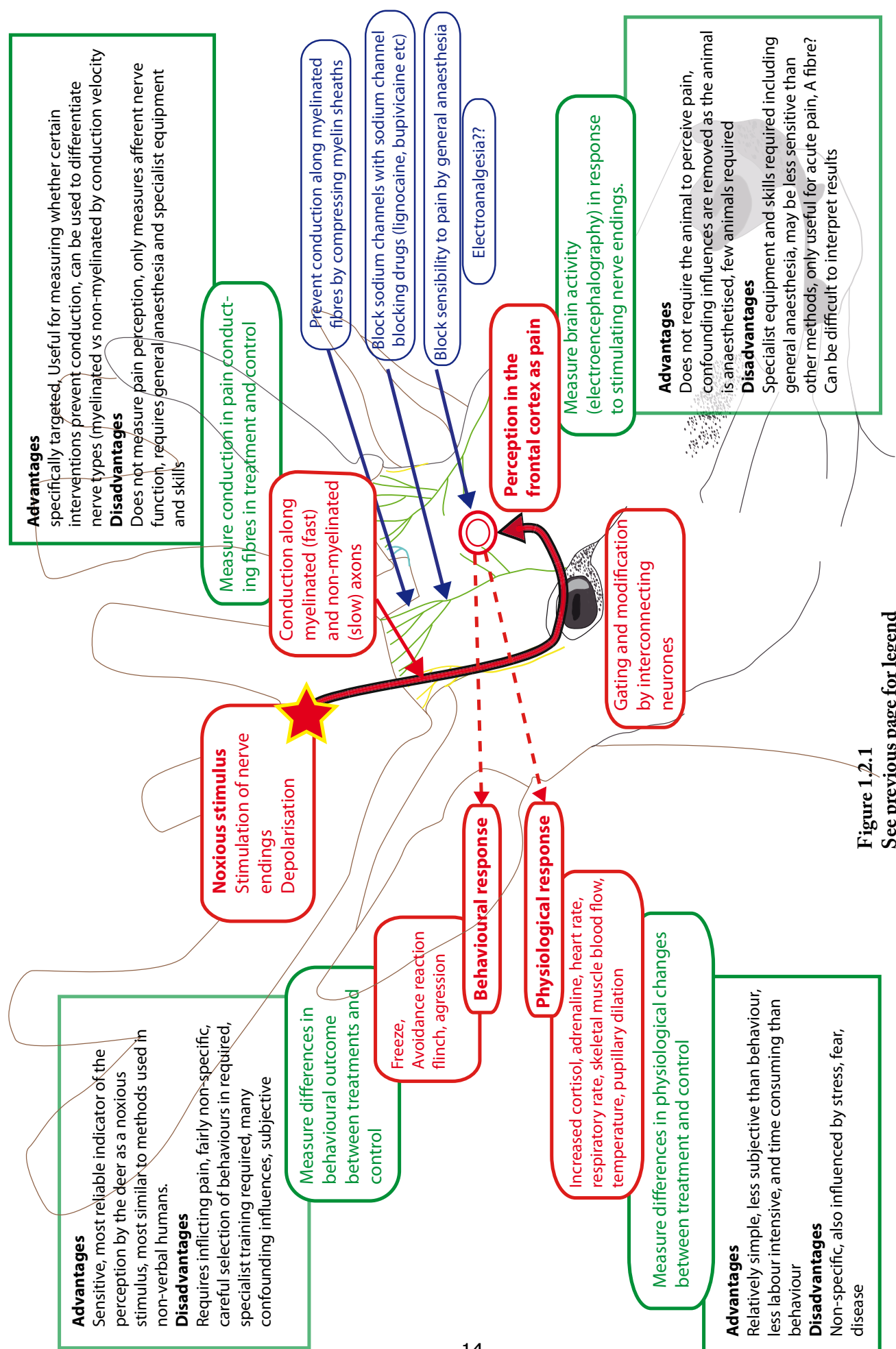


Figure 1.2.1
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The antler has been shown to contain nerves with both large diameter myelinated and small diameter unmyelinated fibres (Varcek, 1955; Woodburry et al, unpublished) which may represent A delta and C fibres respectively. Nerves have only been identified close to the skin of the antler and not within the core structure and it had been postulated that the principal function of these nerves is nociception for the protection of the growing antler (Wislocki and Singer, 1946, Varcek, 1955, Rayner and Ewen, 1981). Without further evidence, it should be assumed that both types of pain fibres are present and analgesia should be provided that is likely to prevent conduction in both A delta and C fibre types simultaneously.

1.2.2 Methods of assessing effectiveness of analgesia

In order to determine whether an analgesic is effective it must first be determined that pain exists and that the analgesic abolishes that pain. Pain needs to be identifiable and, ideally, quantifiable.

The International Association for the Study of Pain (www.iasp-pain.org) has the following definition of pain: "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". Margo McCaffery (1968) gives a more subjective definition of pain as: "Pain is whatever the experiencing person says it is, existing whenever he says it does". Pain is a subjective emotional and psychological experience that is individual to the person experiencing it and therefore difficult for an observer to quantify.

Communication of pain perception

As pain is specific to the individual experiencing it, the most sensitive and accurate way of determining the degree of pain and the efficacy of analgesia is to ask the patient. In human medicine, various pain assessment tools exist that can help patients describe their pain. The numerical rating scale is ranked from 0 (no pain) to 10 (worst pain imaginable). The visual analogue scale is a straight line with the left end representing no pain and the right end of the line representing the worst pain. The categorical pain scale has four categories: None, mild, moderate, severe. The pain faces scale uses six faces with different expressions on each face. For each of these, patients is asked to choose the number, position, category or face that best describes how he or she is feeling.

These methods are fairly straightforward for patients that are able to convey their experience verbally or rank their level of pain on a visual scale. For patients that have difficulty in communicating because they are too young, aged, disabled or because of cultural reasons or fear, determining the level of pain experienced is more difficult (e.g. Stevens and Franck, 2001). In animals, it is even more challenging (Barnett, 1997; Otto and Short, 1998). For pets such as cats and dogs that experience acute pain, a withdrawal response, vocalisation or aggression may be evident and for chronic pain, the owner may detect subtle changes in behaviour. Holton et al (2001) have developed a composite scale for assessing pain in dogs based on behavioural observations. For farmed animals and especially those of flighty nature such as deer, these changes in behaviour may be quite difficult to detect and only severely painful conditions involving loss of function may be

noticeable such as lameness associated with severe limb infection or fracture. Alternative indicators of pain must therefore be used in these species and in all cases these will be indirect ways of estimating the degree of distress experienced by the animal.

Deer present a particular challenge because of their stress-related response as a result of handling and physical restraint (Woodbury et al, 2001) and between animal variation in the nature and magnitude of this response. This might mask or confound response to painful manipulations. Various methods have been used in deer and the validity of these have been reviewed by Johnson (2003) and Haigh et al (2001) and will be discussed here. The methods used in this thesis were restricted to behaviour and electroencephalography but alternative methods that have been used in deer by other researchers are also discussed here.

Behaviour

Observation of behaviour is probably the most widely used method of determining a pain response in farm and laboratory animals. It is also the only method readily available to the owners of animals, including pets, to predict the emotional state of their animals. Behavioural responses could be considered more closely related to verbal responses from humans that are able to communicate their emotions directly than indirect physiological responses or EEG recordings.

The type of behavioural analysis used in research varies depending on the nature of the noxious stimulus. For acute pain such as velvet antler removal, an immediate withdrawal response at the time of the procedure is usually recorded (Matthew et al, 1992; Matthews and Suttie, 2001; Wilson et al, 1999, 2000; Bartels, 2001; Woodbury et al 2001, 2002; Webster et al, 2001, 2003) whereas for painful procedures of a longer duration such as post surgical pain or chronic pain, changes in behavioural patterns over a longer period of time are generally used (e.g. Matthews et al, 1990; Pollard et al, 1991).

Behavioural responses can tend to be non-specific depending on the behaviours included in the study, the nature of the noxious event and the experimental setting and background stimuli. This is especially the case in deer that often demonstrate extreme behavioural response to capture and restraint which can be difficult to differentiate from the behavioural response induced for the stimulus being tested.

Seksel (2001) considered that, interpreted with care, behavioural and physiological parameters can be used effectively to assess pain induced distress in animals.. Seksel described the following four purposes of behavioural responses to pain that enhance an animals chance of survival:

1. Those often autonomic responses that protect the whole animal or part of it (eg withdrawal reflexes)
2. Those that minimize pain and assist healing (eg lying down)
3. Those that are designed to elicit help or stop other animals (including people) from inflicting more pain (eg communicating vocally, by posture or by other means including smell)
4. Those that induce learning and by modifying an animals behaviour, enable it to avoid recurrence of the noxious experience.

It is important to carefully select the behaviours being analysed. A behaviour that occurs only when animals experience pain is a more sensitive index than a behaviour that also occurs in pain free animals. Behavioural scoring for the majority of velvet related studies have followed a rather broad and non-specific set of criteria as described below.

Acute Withdrawal response scales used in velvet removal studies

Matthews et al (1992) developed a behavioural response scale (0 to 9) to electrical stimulation of the velvet antler of 10 stags by incrementally increasing the voltage applied to the antler and recording the behaviour score on a predetermined scale. The same method was then used to determine the difference in behavioural response to antler electrical stimulation after administering lignocaine as either a ring block around the base of the pedicle or individual nerve blocks at two sites. Score one and two represented head movements alone, scores three to seven included neck and shoulder displacement and scores eight and nine involved whole body displacement. Variation between animals was noted in both the minimum current required to elicit a behavioural response and the upper threshold current that resulted in a highest level behavioural response. When the behavioural response was recorded against the percentage of the maximum threshold, the relationship between behaviour score and current was significant ($R^2=0.88$) and appeared to be roughly parametric. Means only were shown graphically and variance was not shown so that it is difficult to gauge the true relationship in these data. It was not stated whether the person scoring the behavioural response was blind to the stimulus intensity but presumably they were not as the intensity was increased incrementally and then decreased incrementally and was not randomised. This scale was also used by Matthews et al (1999a) to test the efficacy of electroanalgesia. Matthews and Suttie (2001) used this scale to compare lignocaine ring blocks, lignocaine local nerve site blocks, electroanalgesia, compression with a *NaturO*TM ring and compression with high pressure compression bands on the behavioural response of stags to electrical stimulation of the antler. The results concurred with previous findings that the minimum stimulus required to elicit a behavioural response was highly variable between animals but once that threshold was reached, a hierarchical order of increase in behavioural score was seen with increasing stimulus intensity. A simplified version of this scale (using a 0-4 point scoring system) was used to assess the effect of compression analgesia on a behavioural response to electrical stimulation of the antler (Webster et al, 2002, 2003). Grades using this system were defined as: 0= no reaction; 1 = slow movement, not associated with stimulus; 2 = rapid reaction or flinch of the head; 3 = sharp reaction or jump of whole body; 4 = major struggle or panic. Scores 0 and 1 were grouped as non-aversive and scores 2, 3 and 4 were grouped as aversive.

Wilson and co-workers (1999) also developed a behaviour scale using electrical stimulation of the antler at different voltages, and observed behavioural responses with or without analgesic drugs. On that scale 0= no movement, 1= slight head movement, flinch, muscle tension, eye or facial movement, 2= Head movement or shake, moderate attempt to avoid stimulus by head movement only, 3= Whole body struggle “flight response” and 1, 2 and 3 were grouped as indicating a pain response.

All animals given electrical stimulation to the velvet antler without local analgesia demonstrated a

pain response. 100% of animals given a high dose ring block did not respond to the same electrical stimulation one minute after treatment. These observations lend to the following conclusions. 1. Velvet antler is pain sensitive tissue. 2. Effective analgesia can be achieved by subcutaneous injection of local anaesthetic. 3. Behavioural responses are likely to be valid indicators of pain. The behavioural criteria used in this study were also used by Wilson et al (2000), Bartels et al (2001), and Woodbury et al (2001, 2002) in measuring a behavioural response in deer that were provided with either electroanalgesia, compression analgesia or various doses of different local anaesthetic prior to antler removal.

The above studies, with the exception of Woodbury et al (2001 and 2002) have all used electrical stimulation of the antler as a proxy for the noxious stimulus associated with cutting the antler with a saw. In addition some of these studies have included observations during actual saw cutting. Using electrical stimulation of the antler has the advantage that the stimulus can be applied remotely without direct contact from the operator, thus reducing the chance of the stag responding to physical contact. In addition, repeated stimuli can be applied and if the same response is seen every time it confirms that it is truly in response to the electrical current. The behaviours demonstrated in response to electrical stimulation may be different to those that result from cutting of the antler with a saw.

In most cases there has been greater response to cutting of the antler with a saw than to electrical stimulation (Wilson et al, 2000; Webster et al, 2003) and it has been suggested that this may be due to non-specific movements caused by human contact and/or vibration of the saw. Wilson et al (2000) tested for the effect of handling and vibration against the antler by holding a piece of wood against the antler and cutting it with a saw. Up to 58 animals were used (exact number used for wood cut test not specified) and 8 of these responded. However, the authors do not say how long after local anaesthetic administration this was performed or what site and dose of lignocaine was used in the stags that reacted to wood cut tests or what behavioural score was demonstrated by the deer that reacted to the wood cut test. This demonstrates potential non-specificity in the behavioural response when the antler is being cut and suggests that pain responses may have been overestimated when field studies have been conducted. Alternatively, the noxiousness of cutting the nerve endings with a saw may be considerably greater than an electrical stimulation across the surface of the antler skin.

When Webster et al (2003) tested the effects of compression bands compared to lignocaine ring blocks on stags on commercial farms they found that a proportion of animals still demonstrated a behavioural response to antler cutting with both forms of analgesia, despite these preventing a behavioural response when tested on the research farm using electrical stimulation.

None of the above authors have taken account of the difference in temperament of the stags or environment on different farms, although Morrow and Matthews (1998) did make note of this in their report. Morrow and Matthews (1998) used a behavioural scoring system of 0 to 3 when assessing velvet removal practices by farmers in the field. In that study scores 0 (no movement) and 1 (flinch of head or body) were combined and regarded as indicative of little or no pain response and scores 2 and 3 (exaggerated or prolonged withdrawal movements of head or body or prolonged struggling movement, jumping or escape from restraint) were combined as indicative

of a pain response. These authors found significant differences between farms, operators and supervising veterinarians. These were most likely to be due to the technique used for injecting local anaesthetic that was taught to farmers by their supervising veterinarians. Some properties were also observed to have a large proportion of stags that struggled a lot, were agitated and poorly restrained, whereas other farms had quiet deer that were well restrained. It was noted that where deer were highly agitated, more of them reacted to antler cutting. It may be that at least some of these animals demonstrated a grade 2 or 3 behavioural response to handling and stress rather than failure of analgesia.

Pollard et al (1992) used a slightly different approach to scoring behaviour of stags during and after velvetting to help assess stress level associated with velvet removal over and above routine handling. Here, struggles were counted as movements of the head or limbs not followed by another movement for 5 seconds. Stags being velvetted struggled more frequently during restraint in the crush than controls, however, there was no significant difference between the number of struggles made during actual antler removal and the control group. This study also found that velvetted stags shook their heads and flicked their ears more than stags that had not been velvetted. These behaviours in addition to whole body responses may be more specific indicators of a response to velvet removal. This is the only study found in which such specific behaviours were included.

Statistical analysis and averaging of behaviour scores

As pain is an individualised experience, the same noxious stimulus applied to two different animals is likely to be perceived differently by each of them resulting in different behavioural responses. Even using a numerical scale to rank the behavioural response, the data are unlikely to be either parametric or normally distributed. Statistical analysis of such data should be kept simple. In most of the studies described above, the outcome has been reduced to a binomial statistic. Either the stag showed a response to the stimulus or did not. Woodbury et al (2001) used a 0-3 behavioural scale in an experiment comparing local anaesthetic and electroanalgesia, and in another study (2002) comparing local anaesthetic with compression bands. In the former study, these scores were averaged to give a "pain score" and compared between the two different treatment groups using a sign-ranked Wilcoxin test (A non-parametric test for comparison of medians). In the latter study, median behavioural responses were compared using a Mann Whitney test, despite 10/16 animals in one group having a 0 behaviour score. There is insufficient justification for this as the only evidence that has been presented to show that a "larger" reaction equates to a more aversive procedure used increasing intensity of electrical stimulation (Matthews et al, 1992). Furthermore, it is meaningless to average a pain reaction score because of the between animal difference in their threshold for pain and the type of reaction that an individual animal will show. In fact the results presented by Woodbury et al (2001) in a bar graph suggest that the mean score for deer treated with local anaesthetic was approximately 0.75 and for electroanalgesia approximately 1.25. As the standard error in these data is likely to be high, it would be probable that a significant difference between treatments would not be shown using mean behaviour scores. However only 5/16 animals showed a behavioural score greater than 0 in the local anaesthetic group while 11/16 responded at a score of 1 or greater in the electroanalgesia group. These proportions alone indicate that the data are not gaussian and although non-parametric tests were used, which should always

compare medians, means were reported in the paper. The median score in the local anaesthetic group would have been 0. Presenting the data in this format is therefore misleading. In the second study by Woodbury et al (2002) the relative risk of a behavioural response for the two different treatments was compared. This is more likely to represent a valid and useful comparison given the nature of the data and assuming a binomial outcome is used.

Behaviour ethograms

A behaviour ethogram is the catalogue of behaviours demonstrated by an animal. Each behaviour is labelled in objective terms describing the physical or vocal display without reference to the purpose of the behaviour. A large number of relatively specific behaviours can be documented and computer software is usually used to record and analyse the patterns of behaviour before, during and after the treatment of interest has been applied. Behaviours are divided into events (behaviours of short duration, e.g. flick ears, stamp foot) and states (behaviours that describe the body position or activity of the animal e.g. lying down, walking, grazing). Live scoring can be used whereby an observer watches continuously and records behaviours as they are seen, or video recording can be used. Some behaviour analysis software allows for video linkage e.g. "Observer" (Noldus Information Technology).

Using behaviour ethograms increases the objectivity of data collection and analysis and allows for retrospective determination of the most appropriate behaviours to use for a particular treatment. To increase the objectivity of the analysis a technician can be trained to identify and score behaviours while blind to the treatment. Roughan and Flecknell (2006) found that by training observers to record specific objective post operative behaviours in rats, the accuracy with which observers could estimate the degree of pain or analgesia supplied was greatly improved over an untrained subjective assessment regardless of the operators previous experience with this species. This indicates that objective behavioural scoring may be a valid method of assessing post surgical pain, and that behaviours specific to the type of noxious stimulus can be identified. Molony et al (2002) found that recording specific behaviour frequencies resulted in accurate allocation of lambs to one of six different castration treatments. The accuracy was increased from 79% to 91% when the number of pain severity categories was reduced from six to three. These authors used principal components analysis to determine the variables that were most useful. The behaviours that are chosen for analysis appear to be very important to the validity of the results. Dinniss et al (1999) were able to differentiate behaviours specific to different methods of castration in lambs and suggested that different methods of castration could not be reliably compared using the same behavioural types. Lambs of different ages also appear to show different patterns of behavioural changes to the same noxious stimulus (Thornton and Waterman-Pearson 2002).

A limited number of studies have described post operative behaviours in stags that were and were not velveteed. Pollard et al (1991) used an ethogram that included a large number of behaviours post velveting in one-year-old stags. Most of these did not differ significantly between treatment groups, except that stags that were velveteed shook their heads more, flicked their ears more, groomed themselves more, spent less time grazing during the first 3.5 hours and more time grazing between 3.5 and 9 hours post treatment. These researchers also studied the duration of behavioural effects and looked at the effect of giving a long acting analgesic on behaviour post

velveting (Pollard et al, 1992). Matthews et al (1990) studied the effects of velveting on various behavioural parameters and found no difference in the posture or activity of stags that were or were not velveted.

Chapter 3.4 of this thesis examines the effects of *NaturO*TM rings on the behaviour of yearling stags. The potential pain indicative behaviours to an insult that is likely to be slower in onset and prolonged over a period of time, such as tight tourniquet or *NaturO*TM rings have not been described in peer reviewed literature for deer. It is unclear what the most appropriate behavioural observations are for the investigation of the noxiousness of compression in stags. Furthermore, it would be important to differentiate between behaviours that indicate discomfort and those that are demonstrated because the stag is aware of an unusual sensation (or perhaps lack of sensation) about the pedicles and antlers.

Preference testing

Another potentially useful way of determining how aversive certain procedures are is to use preference testing. Pollard et al (1994) used a race with a choice of two pens at the end. Each pen contained a “treatment” being either nothing in the pen, an object or a person crouching or standing up. Seven out of 8 stags consistently chose the empty pen, irrespective of which side it was on (left or right) and this was repeated after switching to the opposite pen. These authors also tested whether stags that were velveted took longer to enter the crush than stags that had not been previously velveted. They did not find any difference.

Effects of tranquilisation, sedation and general anaesthesia on behaviour during velvet antler removal

Tranquilisation with the phenothiazine, Zuclopenthixol acetate has been shown to reduce handling stress in wapiti (Read et al, 2000) and improve the specificity of behavioural response to velvet antler removal. In examining the difference between lignocaine and electroanalgesia (Woodbury et al, 2001) and lignocaine versus compression bands (Woodbury et al, 2002), heart rate, blood pressure and behavioural responses were measured in wapiti bulls that were previously tranquilised with 1mg/kgBW of zuclopenthixol acetate 18 to 24 hours before being physically restrained for the experimental procedure. Under this tranquilisation treatment, the animals were reasonably alert and sensitive to handling pressure but lacking in the usual alarm or sympathetic response to human touch and at no time was a panic response detected during handling or antler removal yet behaviours and physiological changes were easily detectable.

It has been suggested that sedation of deer with xylazine may blunt the behavioural response to velvet antler removal in the face of insufficient analgesia (Morrow and Matthews, 1998). Wilson et al (1996) observed withdrawal responses in most deer during velvet antler removal following sedation with up to 0.75mg/kg equivalent of xylazine in the combination drug “Fentazin” and up to 0.7mg/kg of xylazine alone. These doses all resulted in recumbency in the deer and showed that even in heavily sedated deer a behavioural response to cutting the antler without analgesia can be detected.

Even under general anaesthesia, it may be possible to see a behavioural response to a painful stimulus. In a study on lightly anaesthetised stags comparing lignocaine ring block, high pressure compression band and no analgesia, eight out of nine stags in the control group showed somatic movements during the removal of the antler (Woodbury et al, 2005).

Cerebral processing - Electroencephalography

The electroencephalogram (EEG), first recorded in rabbits and monkeys by Caton in 1875 and later developed in humans by Berger in 1924 (Haas, 2003; Ormerod, 2006) is the record of changes in potential difference (voltage) between two electrodes placed on the surface of the brain, dura or skull over separate regions of the cerebral cortex. The techniques used to record EEG and its application as a tool to assess pain in animals have been reviewed (Murrell and Johnson, 2006). EEG is considered to be a useful tool for measuring pain in farm animals (Barnett, 1997).

A large number of studies investigating electroencephalography in animals have now been published. While the majority have focused on changes in response to different anaesthetic protocols, (Antunes et al, 2003a, 2003b; Taylor and Vierck, 2003; Johnson et al, 1998, 1999) a number have been carried out in different species to investigate the effects of visceral or somatic noxious stimuli on the EEG. Very few of the published studies have used EEG as a tool to compare the relative noxiousness of different treatments or effectiveness of analgesics in animals.

EEG frequency spectrum changes in response to an acute noxious stimulus have been investigated in horses during castration (Murrell et al 2003), lambs in response to mulesing, castration and tail docking (Jongman et al, 2000), tail pressure in cats (Taylor and Vierck, 2003), calf scoop dehorning (Gibson et al, 2007), electrical, mechanical and thermal stimulus of the tail of rats (Murrell et al, 2007), repeated balloon inflation in the GI tract of rats (Murrell, 2007), repeated stimulus of the leg of sheep (Morris, 1997) and application of high tension bands in deer (Johnson et al, 2005). The latter is the only study that has been found that used EEG in deer.

The EEG waveform generated is thought to represent the sum of electrical vectors within conducting tissues affecting the pathway between the two electrodes (Murrell and Johnson, 2006). The electrical vectors measured using this method are all far-field (at some distance from the electrodes) and can be influenced by strong electrical generators distant from the brain, for example, the heart or muscular twitching. Placing a third earth electrode between the rest of the body and the area of interest can help to focus the measurements. Raw EEG is difficult to interpret due to the complex nature of the waveform generated. Fast fourier transformation (Welch, 1967) allows the theoretical sine waves of the different frequency bands that contribute to the total EEG to be separated and their amplitudes quantified. It is changes in the relative contribution of these components to the overall waveform in response to different conditions that has been the major focus of studies until now. The commonly used parameters in animal models are the total power (TP) (Total area under the frequency *vs* power curve), median frequency (F50) (frequency below which 50% of the total power is generated) and 95% spectral edge frequency (F95) (frequency below which 95% of the total power is generated). Some authors have adopted frequency bands used in human EEG analysis while others have divided the frequency spectrum broadly into high frequency and low frequency components (Taylor and Vierck, 2003). These divisions may not be

appropriate for all species and the relationship between different sections of the frequency spectrum and stimulus types or states of awareness are not yet clearly defined.

Recording EEG in a lightly anaesthetised animal, it is possible to isolate the response to pain from that of other stressors because the animal is unaware of its surroundings. Using this method, a control group that has not received analgesia can be included because under general anaesthesia, the animal is insensible to pain even if the pain pathway from the receptor to the brain is still intact. The inclusion of a “positive” control group is largely precluded in awake animals due to the welfare implications of inflicting a noxious stimulus likely to elicit the sensation of pain for experimental purposes.

Nerve conduction studies

Measuring nerve conduction is a well described method commonly used in human diagnostics (Kimura, 1984). Direct measurement of conduction along nerve fibres is a potentially useful method for determining the efficacy of different analgesic methods. This method has been used to study the effects of nerve compression on conduction in other species (Rydevik and Nordborg, 1980) but has not been used to study analgesia for velvet antler removal in deer. An electrical stimulus is applied distal to the site of nerve block and an electrical current is detected proximally. The time taken between the stimulus and the response can be used to determine the speed of conduction along the nerve fibre and detect a difference between slow conducting unmyelinated fibres and fast conducting myelinated fibres. Repeated measures can be made on the same animal allowing the animal to act as its own control. General anaesthesia should have minimal effect on the conduction along an individually stimulated nerve so that repeated noxious stimuli can be applied without the animal experiencing the pain. This method is highly controlled and specific with limited background noise or confounding. It only measures conduction in that nerve and does not provide any information about the noxiousness of the stimulus as perceived by the animal. This method has not been used in any of the experiments in this thesis.

Pain - Stress response

Stress and pain are closely related (Selye, 1936). Animals and humans under stress have an altered pain perception and pain induces a stress response. The theory is that physical injury poses a threat to the homeostasis of the animal and that the response is designed to maintain homeostasis through avoidance of further injury. The concept of homeostasis or “milieu intérieur” was defined by the French physiologist, Claude Bernard (1813-1878) and the response, often referred to as the “fight or flight” response, was coined by Walter Bradford Cannon (1871-1945). The link between pain and stress has been described by many authors e.g. Melzack (1999), Tsigos and Chrousos (2002) and is largely based on the work of Selye (1936) who describes the stress response as being rather non-specific to a range of different insults. The injury-pain-stress response is briefly described as follows:

Within seconds of physical injury, cytokines are released (e.g. gamma interferon, interleukins 1 and 6, tumour necrosis factor). These enter the blood stream and travel to the brain within

minutes. These cytokines can activate neurones that send messages to the brain, particularly the hypothalamus. At sufficient levels of injury the sympathetic nervous system is activated. Adrenaline is released from the adrenal glands and the locus coeruleus/noradrenaline system is activated setting up the body for immediate response to bodily threat. Increased sympathetic tone results in increased cardiac output and respiration and glucose availability to the muscles and brain with decreased blood flow to non essential organs.

Perception of injury or pain activates the hypothalamic-pituitary-adrenal (HPA) axis. Corticotropin releasing hormone is released from the hypothalamus and travels via the blood stream to the pituitary gland, causing the release of adrenocorticotrophic hormone (ACTH). The ACTH then activates the adrenal cortex to produce cortisol. Cortisol induces gluconeogenesis to raise blood glucose levels and is essential for many body functions. Following pain, glucocorticoids provide analgesia and have anti-inflammatory effects but also suppress the immune system, suppress reproduction, cause catabolism of muscle, fat and carbohydrate and result in gastrointestinal ulcers, muscle weakness and brain dysfunction if levels are increased for prolonged periods. Thus the whole body state is set up for emergency response to severe injury. Both the noradrenaline and HPA systems are inter-related and under the influence of other brain centres such as the mesocortical/mesolimbic system, amygdala/hippocampus and arcuate nucleus.

It is important to consider that while there may be some differences in the stress response dependant on the nature of the stressor, the response is largely non-specific as demonstrated by Selye (1936). An acute painful stimulus may induce changes in the HPA axis that are similar to those induced by other stressors such as chase, capture, handling, aggression, hunger, disease or concurrent injury.

Cortisol

Changes in plasma cortisol concentration have proved to be a useful tool for determining the effect of analgesics on dehorning pain or distress in calves (Petrie et al, 1995; Mellor and Stafford, 1997; McMeekan et al, 1998; Stafford et al, 2003; Stafford and Mellor, 2005) and castration and tail-docking in lambs (reviewed by Mellor and Stafford, 2000). These studies have shown differences in cortisol concentration related to the severity of the pain insult and the amount of analgesia that is provided before surgery. In lambs a grading system based on cortisol concentration can be applied (Mellor and Stafford, 2000).

The measurement of cortisol concentration to assess pain-induced stress in response to velvet removal in deer has produced variable results. Cook and Schaefer (2002) found that wapiti stags that were velveted without analgesia had higher salivary cortisol levels than those velveted with either lignocaine or electroanalgesia and that salivary cortisol was higher on the second day of sampling than the first. Matthews and Cook (1991) found no difference in cortisol concentration between animals that were velveted using local anaesthetic only and those that were handled but not velveted. The authors concluded that velvet removal was not a significant source of stress as it did not increase cortisol levels above handling. Another possible explanation for this finding is that maximal cortisol concentrations are developed in response to the stress of handling alone and no further rise in response to velveting is possible. Animals that were in hard antler at the time

of removal had lower cortisol levels and animals that were sedated with xylazine also had lower cortisol levels. Xylazine suppresses noradrenaline release from the locus coeruleus (Benarroch, 2009) and may have an effect on the secretion of cortisol. Sedation may also decrease the animal's awareness of handling stress and thus reduce the psychological component of the stress response. Lower cortisol concentration during removal of antlers in the hard antler state may reflect the physiological state of the animal at that time of the year when there is a seasonally determined increase in testosterone.

Woodbury et al (2001) measured serum cortisol levels from wapiti bulls that had been previously sedated with zuclopenthixol and had their velvet antlers removed after either lignocaine ring blocks or electroanalgesia and in another study in wapiti bulls that had their velvet antlers removed after either lignocaine ring blocks or a high pressure compression band (Woodbury et al, 2002). Arterial blood samples were used and were drawn prior to administering the analgesia and 3 minutes after cutting the antler. There was no significant difference in cortisol levels between pre and post antler removal within or between treatment groups in either of these studies despite differences in heart rate and behaviour and in some cases in blood pressure.

Webster et al (2003) measured salivary cortisol during application of lignocaine ring blocks, compression bands, ear tags and restraint only from red deer stags (8 to 9 per treatment). Not all of the results were recorded in the publication but the authors reported that deer in the lignocaine group had higher mean salivary cortisol levels than other treatments (time not stated) and that there were no differences between treatments in the change in salivary cortisol levels from the start to the finish of the experiment. They did not state whether there were differences within treatments between these two time intervals. These results show that the additional manipulations of applying high pressure compression bands, lignocaine ring blocks or ear tags did not raise cortisol levels any more than restraint in the crush alone.

Heart rate

Increases in heart rate have been recorded in deer in response to electrical stimulation or cutting of the sensitive velvet antler (Matthews et al, 1992; Woodbury et al, 2001; Woodbury et al, 2002).

Matthews et al (1992) used ECG to record heart rate during electrical stimulation of the velvet antler without analgesia and following analgesia with either a lignocaine ring block or two individual nerve blocks. These authors found a curvilinear correlation between the mean heart rate of 10 deer and the percent of the current that elicited the maximal behavioural response. When electrical stimulation was applied at various times after application of a ring block in seven stags, the heart rate was higher at times when local anaesthetic was not present (prior to injection and 24 hours after injection) than at times when local anaesthetic was present (5 and 30 minutes after injection). In this study, the variation between animals was not shown and it was not stated whether the differences were statistically significant.

Woodbury et al (2001) recorded the heart rate in wapiti bulls comparing lidocaine (lignocaine) analgesia and electroanalgesia for antler removal. The stags had been previously tranquillised with zuclopenthixol and were restrained in a hydraulic crush for administration of lidocaine and antler

removal. Heart rates were recorded prior, during (one recording) and 4 minutes post lidocaine application, immediately post antler removal and at one, two and three minutes post antler removal. Stags in the lidocaine treated group maintained reasonably stable heart rates of around 80bpm throughout the procedure despite the fact that five out of sixteen of these animals showed a behavioural response believed to indicate a pain reaction during antler cutting. Animals subjected to electroanalgesia showed a slight, but not statistically significant increase in heart rate from baseline at one time point during application of the electroanalgesia. This was significantly increased to a mean of about 95bpm and 100bpm at 4 minutes of analgesia (immediately before cutting) and at cutting respectively. Heart rates in this group dropped back towards 90bpm after velvet removal, but remained higher than the lidocaine treated group. It appears from this data that the increase in heart rate was in response to antler cutting rather than the application of the electroanalgesia. In a similar study comparing lignocaine ring blocks and high pressure compression bands for analgesia, wapiti bulls that had compression bands applied had an increased heart rate during application of the bands and both treatments groups had increased heart rate immediately after antler removal (Woodbury et al, 2002). The increase in heart rate may have been in response to close human contact during application of the analgesic or during antler cutting rather than a response to pain.

Blood Pressure

Blood pressure increases in response to stress by increased cardiac contractility and increased peripheral vascular resistance due to vasoconstriction. These effects are induced mostly by increased sympathetic tone through the release of adrenaline from the adrenal medulla and activation of the locus coeruleus/noradrenaline system. Blood pressure has been recorded in a few studies related to velvet removal in deer.

Woodbury et al (2002) measured blood pressure in wapiti bulls that were sedated with zuclopenthixol and had antlers removed following local analgesia with either electroanalgesia or lignocaine ring blocks and in another study following either high pressure compression bands or lignocaine ring blocks (Woodbury et al, 2001). Stags were restrained in a hydraulic “chute” and a catheter was placed in the medial auricular artery and attached to a pressure transducer. There was no significant change in blood pressure in response to application of any of the analgesic treatments or antler cutting.

Infrared Thermography

Body temperature rises in response to stress as a result of increased sympathetic tone and direct effects on the hypothalamus temperature set-point (Briese and Cabanac, 1991; Oka et al, 2001).

Webster et al (2003) found increases in maximum orbital infrared values in deer that were restrained and treated with either a lignocaine ring block, high pressure compression band, ear tagging or restraint alone when the temperature at the start of restraint was compared to the temperature at the end of the procedure. The increase ranged from 0.56 to 0.97 °C with stags treated with lignocaine ring blocks showing a significantly greater change in temperature than those re-

strained or treated with an ear tag although the difference in absolute temperatures between these treatment groups was not significant. Cook and Schaefer (2002) also demonstrated increases in radiated heat loss in wapiti bulls following velveting and in that study, the increase was significant in stags treated electroanalgesia or no analgesia but not in those treated with lidocaine prior to velvet antler removal. It is not known what the increase in body temperature due to the stress of handling will be in individual deer and whether a painful procedure in addition to handling would increase body temperature further. It is probable that a stress induced raising of the hypothalamic thermal set-point would have a maximum limit. The equipment used by the AgResearch team is quite expensive and specialised (M. Stewart, pers. comm.). Convincing evidence that this method increases the sensitivity and specificity of identifying a pain response above other methods would be required before investment was made in using infrared thermography.

1.2.3 Methods of preventing pain during velvet antler removal

Injectable sodium channel blockers

The commonly used local anaesthetic drugs in veterinary medicine include lignocaine (lidocaine), bupivacaine and mepivacaine with lignocaine used in highest quantities. The earlier amino-ester anaesthetics are now largely superseded due to short duration of action, perceived poorer efficacy and potential for adverse side effects although chlorprocaine showed potential for velvet removal (Wilson, *unpublished*).

Lignocaine (Figure 1.2.2), 2-(diethylamino)-N-(2,6-dimethylphenyl) ethanamide is an amide anaesthetic discovered by Nils Löfgren in 1943 and brought into clinical use by Torsten Gordh from 1948 (Gordh et al, 2010). Lignocaine is used extensively in human and veterinary medicine as a local anaesthetic and antiarrhythmic and is the only form of local anaesthetic used routinely for velvet antler harvest.

Figure 1.2.3 (courtesy of Dr. Paul Chambers) shows step-by step diagrams of the action of lignocaine. The physiology and pharmacology of amide local anaesthetic agents has been reviewed by Covino (1981). Most amide local anaesthetics function in a similar way and their speed and

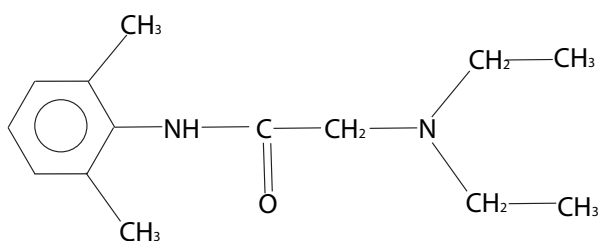
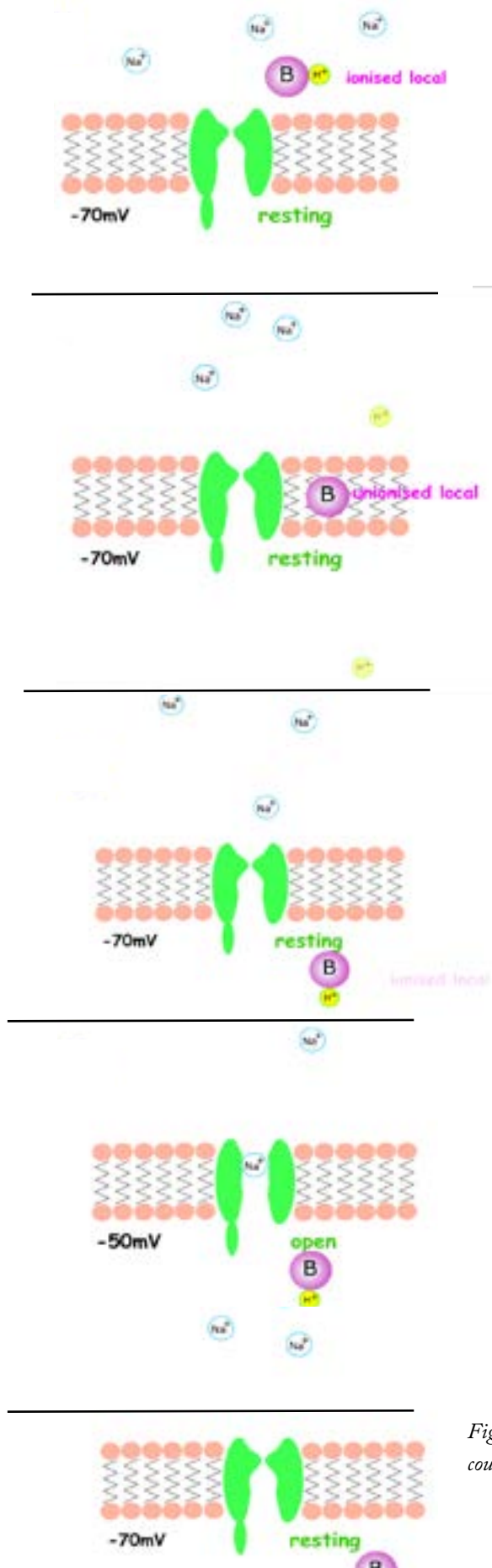


Figure 1.2.2 Molecular structure of lignocaine
2-(diethylamino)-N-(2,6-dimethylphenyl) ethanamide



duration of action are related to their pKa and protein binding properties. The local anaesthetic moves across the cell membrane in the unionised form and acts from inside the cell. Within the cell the drug binds with and blocks fast voltage-gated sodium ion channels when they are in the "open" state, preventing further influx of sodium ions and stopping the propagation of an action potential generated in response to stimulus of the sensory nerve.

The relatively low pKa (7.8) and high percentage of unionised base (25–30%) of lignocaine at pH 7.4 (Joyce et al, 2004) allows it to cross the cell membrane rapidly giving it a faster onset than other amide anaesthetics such as bupivacaine. Lignocaine has a lower receptor binding affinity and is able to move out of the cell easily and thus has a shorter duration of action compared to bupivacaine as demonstrated by Bartels et al (2001).

Analgesia success rate using lignocaine

Data from research environments show up to 100% analgesia success rate (Wilson et al 1999; Bartels et al, 2001). In practice, achievement of analgesia may be more variable (Morrow and Matthews, 1998; Webster et al, 2003; Conway, 2005). Morrow and Matthews (1998) conducted a confidential survey for MAFpolicy that involved observing velvet removal by farmers from 70 stags on 11 farms in the Waikato and Bay of Plenty regions. There was variation noted in the injection sites (8 operators gave injections at the zygomaticotemporal and infratrochlear nerves only, 5 operators injected at these two sites and ad-

Figure 1.2.3 (left) Mechanism of action of Lignocaine. Pictures courtesy of Dr. Paul Chambers, Massey University

ditionally at the “cervical” nerve and 3 operators used a ring block). There was variation between operators in the volume injected (10 to 35 mL per animal), method of tourniquet application, tourniquet efficacy, and wait time (2 min 43 sec to 9 min 2 sec). Variation was also seen in the temperament of the stags (calm and well restrained to highly excited and poorly restrained) and behaviour response to antler cutting (16.7 to 90% effective analgesia). There was consistency between operators who were trained by the same supervising veterinarian in some of these variables and there was a significant effect of the supervising veterinarian on the analgesic success rate of the farmers trained by that veterinarian. Overall, 66.9% of antlers were considered to be analgesic at the time of removal according to the behaviour criteria used in that study. The range of 16.7 to 90% indicated that some operators require significant improvement in their method and that on the other hand, high success rates were achievable.

Webster et al (2003) compared compression analgesia and lignocaine ring blocks on 13 farms and 237 stags and found an average of 0.19% and 0.29% of animals showed a behavioural response during the nick test and antler cutting respectively following lignocaine rings blocks. Three of the farms had 60% or more stags show a behavioural response during antler cutting and one farm had no stags react. The number of stags on each farm was not reported. The 2005 NVSB auditors’ report (Conway, 2005) recorded 85% analgesia success on the first nick test and 93% success on cutting.

There is a need to better define best practice and to identify factors that make some operators more successful than others at achieving analgesia. This was the major aim of the experiment in Chapter 2 and the factors influencing analgesia success that have already been studied are reviewed in detail below.

Injection sites

Figure 1.2.4 shows the anatomical location of the nerves to the antler and recommended positions of injection sites. The site of deposition of the lignocaine is likely to have the most significant effect on the efficacy of analgesia as it can only be effective if it reaches and blocks all of the sensory nerves from the antler. Early recommendations on the most appropriate sites for deposition of local anaesthetic were based on the anatomical studies of Adams (1979) and included blocking the zygomaticotemporal nerve beneath the zygomatic arch and the infratrochlear nerve above the orbital crest.

Woodbury and Haigh (1996) supported this anatomical distribution of local anaesthetic but also discuss the importance of the auriculopalpebral branch of the trigeminal nerve and the necessity for further local anaesthetic placed between the ear and the pedicle. This nerve would not be blocked by placement of two injections at the sites recommended by Adams (1979) who concluded that it was not necessary to block the auriculopalpebral of the facial nerve because it is believed to have motor function only. Woodbury and Haigh suggested that the auriculopalpebral nerve may in fact contain a mixture of fibres, some of them being afferent axons from pain receptors. These authors recommend that in all cases local anaesthetic should be infiltrated in a ring of overlapping subcutaneous injections around the entire base of the pedicle at the angle between the pedicle and the skull. Woodbury and Haigh recommend using a 20g needle, 4cm long and a total

volume of 10-15mL per pedicle.

Research into routes and doses of local anaesthetic for velvet analgesia support the recommendations made by Woodbury and Haigh (1996). Matthews et al (1992) found that fewer stags responded to electrical stimulation of the velvet antler after a ring block than after two individual nerve blocks. (1/12 versus 4/12 showed behavioural response respectively). In a study conducted by Wilson et al (1999) 64 yearling stags were used in 10 different treatment groups (23-24 antlers per treatment) to investigate various routes and doses of lignocaine administration. In this experiment none of the stags given a “high dose” ring block of 15mls per antler responded to electrical stimulation of the antler 1 minute after treatment, whereas all stags given a “low site nerve block” without an auriculopalpebral block responded 1 and 2 minutes after treatment, and 75% still responded 8 minutes after treatment demonstrating that the ring block was more effective than individual nerve blocks of the zygomaticotemporal and infratrochlear nerves only. A further study by Wilson et al (2000) showed that a “high site nerve block” was more effective than a “low site nerve block”. For the high site 21/25 animals did not respond to electrical stimulation of the antler 2 minutes after treatment whereas 15/25 animals in the low site group did not respond. The “high site nerve block” was still inferior to using a “high dose ring block” in that study as 24/25 animals did not respond to electrical stimulation of the antler 2 minutes after “high dose ring block”. Thus, there is convincing evidence that a ring block around the base of the pedicle is effective on a greater proportion of stags than blocking nerves at a more proximal location.

Injection volume or dose

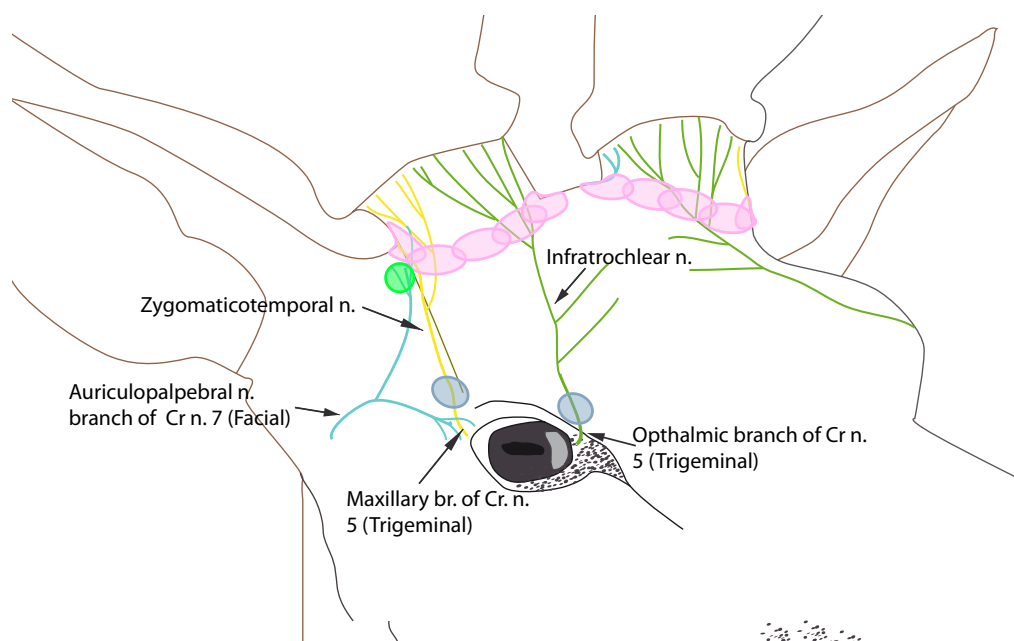


Figure 1.2.4 Recommended sites for placement of local anaesthetic to block nerve conduction from the velvet antler and provide analgesia for velvet antler removal. Local nerve sites, as recommended by Adams (1979) are shown as blue shaded circles with an additional site to block the auriculopalpebral nerve recommended by Woodbury and Haigh (1996) in green and the “Ring block” is shown in pink shaded circles.

The dose rate also appears to affect the outcome. In the study mentioned above by Wilson et al (1999) “Medium dose ring block” (10mL/antler) resulted in about 10% response rate to electrical stimulation and low dose (5mL/antler) resulted in 13% to 48% response, decreasing with time. All groups showed general trend in decreasing response rate with time, demonstrating that post treatment wait time is likely to be important except in high dose ring block which achieved 100% effectiveness within 1 minute.

When comparisons were made during test cut and antler removal (as apposed to electrical stimulation), there were no statistical differences between high, med and low dose ring blocks.

In a further study in 2000 using 50 adult red stags (all greater than 2 years of age) (Wilson et al, 2000) the dose rate for local anaesthetic was more specifically defined. The pedicle circumference was measured and the dose was determined as the number of mL of lignocaine injected per cm of pedicle circumference. Note that the pedicle circumference is not the same as the distance over which the injections are made in a circle around the base of the pedicle as all of these injections are not made on the pedicle itself, but at a more proximal location and thus follow a longer path than the length of the pedicle. Nevertheless, using this criteria to define the dose rate allows for differences in antler size which are likely to be related to the number of size of nerves supplying the antler. High dose ring block (1ml/cm pedicle circumference) was more effective (24/25 showing no response to electrical stimulation of the antler after 2 minutes) than a low dose ring block of 0.4ml/cm (20/25 showing no response) and either a high (21/25) or low site (15/25) nerve block. Also, analgesia may be achieved faster with a high dose ring block.

Based on this research a high dose rate (1mL/cm pedicle circumference) in a complete ring block around the base of the pedicle provided the most reliable and rapid analgesia.

Wait time

Lignocaine takes time to diffuse through the tissues, enter the nerves and block the sodium channels from within the cell, necessitating a wait period between injection of the drug and removal of the antler. Onset of analgesia is rapid following a ring block with lignocaine, mepivacaine or bupivacaine with only small differences between the drugs. Yearling stags ceased showing a behavioural response to electrical stimulation of the antler 15-195 seconds after lignocaine, 15-300 seconds after bupivacaine and 15-75 seconds after mepivacaine was injected as a ring block around the base of the pedicle (Bartels et al, 2001). All seven yearling stags tested by Matthews et al (1992) ceased to show a response to electrical stimulation of the antler by five minutes after injection of lignocaine. Morrow and Matthews (1998) found that farmers removing velvet from their own stags waited between 2 min 43 seconds and 9min 2sec between injecting local anaesthetic and cutting the antler. There was no significant effect of wait time on withdrawal response within this time range with 36.4% (8/22) of stags that had antlers removed less than 4 minutes after treatment showing a withdrawal response compared to 33.1% overall withdrawal responses although this was confounded by local anaesthetic method. Of 18 stags given a “high dose ring block”, (Wilson et al 1999) 3/18 stags responded to electrical stimulation two minutes after treatment and one responded after four minutes indicating that some stags require up to four minutes for full analgesia to take effect. Based on these findings it seems appropriate to apply a 4 minute

wait time to ensure the maximum number of stags have the full effect of lignocaine. Any reaction after this time period is likely to be due to incorrect distribution of the lignocaine rather than not allowing enough time for the drug to work.

Bartels et al (2001) timed the procedure of injection of local anaesthetic, application of tourniquets and changing equipment to determine the time between completion of the ring block and cutting the antler when the process was performed continuously. On no occasion was the antler cut less than one minute after completing the ring block. The authors suggested that analgesia was effectively achieved in most stags by this time and therefore a wait time was not required provided a contingency response was adopted (further wait time) if the animal responded to the test cut. If this recommendation is followed, it is likely that a proportion of animals will be subjected to a painful test cut.

Duration of analgesia

Lignocaine prevents a behavioural response to electrical stimulation of the antler for one to two hours following application (Bartels et al, 2001; Matthew et al, 1992). A longer duration of analgesia is seen following mepivacaine (180-375 minutes) and bupivacaine (195-570 minutes) with combinations of drugs tend to reflect the longest acting drug in the combination (Bartels et al, 2001). In the study by Bartels et al (2001) there was considerable variation between animals (2-3 fold) which may be related to antler blood flow and removal of the drug from the site of administration or individual animal sensitivity to the drug or to the different rates of metabolism in individuals. Duration of analgesia and post-velveting pain control are not subjects of this thesis.

Potential adverse effects

The use of injectable sodium channel blocking agents is not free of negative effects. Potential problems include adverse drug reactions, failure of analgesia, pain on injection and food safety issues. Lignocaine toxicity is rare in all species as evidenced by the lack of reports, with the exception of goats (Smith and Sherman, 2009). No published or anecdotal reports of adverse drug reaction to lignocaine in deer were found.

Aversion to injection, shown by movement of the head and body, has been noted in well handled young stags (personal experience) and increases in cortisol and orbital temperature following lignocaine ring blocks were reported by Webster et al (2003). Other studies (Woodbury et al, 2002; Woodbury et al, 2003; Johnson et al, 2003) have not found changes in heart rate, blood pressure or EEG in response to injection of lignocaine. It is unclear how noxious the injection of lignocaine is to the stag.

Lignocaine and other amide anaesthetics including bupivacaine and mepivacaine have a certain metabolite, 2,6-dimethylaniline that has the potential to convert to several highly electrophilic compounds. These compounds have been found to bind to DNA and other macromolecules and have the potential to cause cancerous changes to the cells. This is discussed further in section 1.4.

Electroanalgesia

Electroanalgesia (EA) involves the application of electrical currents that interfere with signals from pain nerve fibres, preventing the sensation of pain. This method has been tested for velvet removal in the search for drug free methods of analgesia. The general consensus is that the method is neither effective nor humane. During application of EA, Matthews et al (1999a) observed that stags showed eye twitching, eye watering, bulging of the eye, swelling behind the eye and flattening of the ears. Twelve of 17 stags jumped during electroanalgesia. Woodbury et al (2001) described momentary aversive movements and a brief struggle when EA intensity was increased to a new level and persistent movement whenever the animal's threshold was reached along with increased heart rate in EA treated deer immediately after treatment. These observations suggest that the application of EA may be unpleasant to the stag.

Electroanalgesia does not appear to prevent sensation during antler removal. EA did not provide sufficient analgesia to prevent a withdrawal response to electrical stimulation of the antler whereas lignocaine ring blocks increased the threshold at which stags reacted (Matthews et al, 1999a). During antler removal, 11/16 lidocaine treated animals and 2/16 EA treated animals did not show a withdrawal response (Woodbury et al, 2001). Electroimmobilisation was also considered to be an unsatisfactory means of restraint in calves, sheep and pigs (Lambooya, 1985).

Compression

There has been some interest in nerve compression as a form of analgesia with the main driver for this being the desire for drug free methods of velvet harvest to avoid drug residues in the product (Woodbury et al, 2005; Cook et al, 2005). There are two forms of compression used for velvet analgesia. High pressure compression bands are used overseas but not in New Zealand whereas medium pressure compression bands are commonly used on yearling deer in New Zealand.

Effects of compression on nerve function

The effects of compression on nerve function have been widely studied in order to better understand compressive nerve disorders and effects of tourniquet use in people. Compression causes cessation of the action potential in sensory nerve fibres which may be due to ischaemia (lack of blood) or deformation of the nerve axon, or a combination of these effects. The time to onset of action potential failure, order of nerve types affected and likelihood of prolonged structural damage are dependant on the compressive force applied (Dahlin et al, 1986; Fern and Harrison, 1994b; Rorabeck, 1980; Rydevik and Nordborg, 1980) and the length of time compression is maintained (Nitz and Matulionis, 1982). Low pressure (50mmHg) applied to rabbit tibial nerves for 2 hours caused only mild changes in conduction velocity and nerve structural integrity, whereas pressures of 200mmHg applied for two hours resulted in reduced conduction velocity and moderate structural damage that persisted for at least three weeks and at 400mmHg these changes were severe (Rydevik and Nordborg, 1980). Tourniquet pressure of 300mmHg applied over the sciatic nerve of rats resulted in structural damage that was related to compression time and affected

large diameter nerves more profoundly than smaller diameter nerves. Very few changes were seen after 30 minutes, whereas after two hours, damage included axonal shrinkage, splaying of myelin lamellae, Schwann cell hypertrophy and complete rupture of myelin sheaths in some fibres (Nitz and Matulionis, 1982).

Fern and Harrison (2004a,b) conducted experiments to investigate the relative importance of ischaemia and compressive deformation on different nerve types and their conduction survival time. When ischaemia was induced by cardiac arrest, the action potential recorded from various hindlimb nerves in cats failed in slow conducting myelinated fibres from 11 minutes, but persisted for up to 39 minutes in faster conducting fibres. Low pressure balloon inflation directly over the isolated sciatic nerve of cats also resulted in loss of action potential in slow conducting axons earlier than faster conducting axons, whereas high pressure balloon inflation tended to prevent action potentials in faster conducting nerves earlier, indicating that different nerve types may be affected differently by ischaemia or direct compressive forces. The differential effect of compressive force or ischaemia on myelinated vs unmyelinated nerves was even more pronounced with unmyelinated fibres being highly resistant to compression, requiring forces up to 400mmHg for 2 hours before action potentials were affected, but highly sensitive to ischaemia induced by nitrogen inhalation or aortic occlusion (Dalin et al, 1989).

Compression analgesia for velvet removal

For the high pressure compression, the method has been described by Webster et al (2003) as follows: “... consists of a precision manufactured latex band, which is tightened around the pedicle to a specific pressure, in a consistent manner, using a custom-made tool (No-Bull Enterprises, Kansas, USA). A metal clip is crimped around the band to retain pressure, and remains around the pedicle until the antler is analgesic. The antler is then removed above the pedicle and the band is taken off.” The time allowed for analgesia had been 4 minutes (Webster et al, 2003).

Woodbury et al (2002) examined the difference in behavioural and physiological responses during velvet antler cutting in wapiti bulls that had been administered either high pressure compression bands (modified Callicrate Smart bander, No Bull Enterprises, St. Francis, Kansas, USA) or lignocaine ring blocks (1.2mL/cm pedicle circumference). The bulls had previously been tranquilised with zuclopenthixol. The band delivered a stated force of 260 Newtons and was left in place on the antler pedicle for a minimum of 4 minutes before antler removal. A greater proportion of deer that had the compression band on showed a behavioural response during antler cutting compared to deer that had lignocaine ring blocks (13/16 versus 6/16 for compression and lignocaine respectively). Both treatments resulted in an increase in mean heart rates immediately after antler removal. There was also an increase in heart rate during the application of the compression band. The authors concluded that the compression band did not provide as consistent analgesia as lignocaine ring blocks and that the compression band may be painful on application.

In another study using halothane anaesthetised stags (Johnson et al, 2005), EEG was recorded during application of high pressure compression bands or lignocaine and EEG was also recorded during velvet antler removal. Cardiovascular and somatic responses were also recorded and reported separately (Woodbury et al, 2005). Deer that had antlers removed without analgesia had

significantly increased median frequency and spectral edge frequency and significantly decreased total power following antler removal. There was no change in blood pressure or heart rate in these animals but eight out of nine demonstrated somatic movements during antler removal despite being under a light plane of general anaesthesia. Deer that had been given a lignocaine ring block 4 minutes prior to antler removal had significantly decreased median frequency during both the application of the local anaesthetic and velvet antler removal. Spectral edge frequency and total power were also decreased during application of lignocaine but not during antler removal, there were no changes to blood pressure or heart rate and somatic movements were observed in one deer during lignocaine injection and none during antler removal. The EEG changes seen during application of lignocaine are not consistent with a noxious somatic stimulus. Deer that had a compression band applied 4 minutes prior to antler removal had significantly increased median frequency and spectral edge frequency during both the application of the bands and removal of the antlers and had significantly decreased total power during removal of the antlers. This pattern of EEG change was similar to but smaller in magnitude than observed in deer that did not have any analgesia and is indicative of cerebral response to noxious stimulus. In addition, deer that had compression bands had an average increase in systolic blood pressure from the time of application of the bands and 3/9 demonstrated somatic movements during application of the bands and 2/9 during antler removal. These results indicated that the placement of the bands causes a response similar to that induced by a painful stimulus without analgesia and that any EEG response to velvet antler removal is still seen 4 minutes after application of a high pressure compression band in some deer.

In support of the use of compression bands, Webster et al (2003) found that 9/10 animals stopped showing a behavioural response to electrical stimulation of the antler above the level of the compression band at an average of 3 mins and 38 seconds after application of the band. These authors did not describe the response to actual cutting of the antler in that experiment but concluded from the response to electrical stimulation of the antler that analgesia had been achieved. In a follow up study (reported in the same paper) on farms in which 237 stags were velveted by farmers using either compression bands or lignocaine ring blocks, the compression band resulted in fewer behavioural reactions than lignocaine ring blocks during the nick test (0.11 vs 0.19) and during antler removal (0.18 vs 0.29). There was considerable variation between farms and this variation was greater for lignocaine ring blocks than compression. On two of 13 farms approximately 60% of stags showed a behavioural response during antler removal with the compression band while on one farm no animals showed a response. For the lignocaine ring block, one farm had no animals showing a behavioural response and 3 farms had 60% or more showing a behavioural response. This indicated that either both of the methods assessed were inadequate and have a large variation in efficacy or the behavioural criteria used to assess a pain response were not suitable for this type of study across different farms.

On assessing the noxiousness of the application of compression bands, Webster et al (2003) compared the proportion of stags that showed a behavioural response during handling (0/8), compression bands (4/8), lignocaine (6/8) and ear tagging (8/9). The authors concluded that compression bands were less noxious than ear tagging on the basis of the proportion of animals that showed a behavioural response according to the criteria used. This is a questionable comparison as discussed in section 1.2.2. These authors also recorded the salivary cortisol levels and orbital infra-

red temperatures from deer in each of these treatments. Lignocaine tended to result in greater increases in infrared temperatures from capture to release but there were no differences between the treatments in absolute temperature. Salivary cortisol levels were also higher in the lignocaine group (time not stated) but the change in salivary cortisol from capture to release did not differ significantly between treatments.

Webster et al (2003) also found that stags that had compression bands on showed a behavioural response to electrical stimulation of the contralateral antler and to ear-tagging and suggest that this demonstrates that the compression band is not so aversive that it prevents a behavioural response to concurrent noxious stimuli.

There remains disagreement amongst researchers on the humaneness and efficacy of high pressure compression bands for analgesia based on the overall analysis of these results. Webster et al (2005) reviewed the research at that time and concluded that compression analgesia was a safe and effective non-chemical alternative to lignocaine for analgesia for velvet removal in adult stags. The National Animal Welfare Advisory Committee has advised that compression is not an acceptable form of analgesia on the grounds that it may be painful on application and is less effective than lignocaine ring blocks. Currently, high pressure compression is not an accepted form of analgesia for velvet removal in New Zealand.

No treatment (“organic” antler removal)

The proposal for no analgesia or “organic antler removal” (Schaefer et al, 2005) has been discussed by farmers and veterinarians on the “elkmed” online discussion forum (<http://tech.groups.yahoo.com/group/elkmed>), with the argument being that the animals are stressed during restraint and therefore less sensitive to pain. It is also argued that the additional time and handling required to administer local analgesia would increase the stress levels and thus the overall welfare of the stag

would be compromised to a greater degree than “get ‘em in, saw ‘em off, get ‘em out” as quickly as possible. This argument is not acceptable under New Zealand legislation and there is no peer-reviewed literature supporting this view.

1.3 Use of the *NaturO*TM ring for velvet antler analgesia

1.3.1 Spiker velvet removal

Every year, hundreds of thousands of yearling deer are sent to venison processing plants in the spring. This time of the year coincides with the period of rapid growth of the first antler (spike) in male deer. Deer cannot be legally transported if they carry antlers greater than 110 mm in length, as there is risk of injury to themselves or others (Anon, 1994; Basteiaansen, 2003; Wild, 2008). The velvet antler must be shortened before transport or the farmer must wait until the antler has hardened and shed its skin in the autumn. At that time the antler is considered insensitive and can be removed without analgesia. The latter presents an economic disadvantage on many farms supplying the main venison market as it will result in sale of stock after the peak venison price schedule and a requirement to carry these animals through the summer when feed is required for lactating hinds and calves. Stags are also more difficult to handle when they have hard antler due to high testosterone levels and the presence of sharp and dangerous antlers that can injure other deer in the yards and the farmer during animal handling.

Methods of velvet removal used in adult stags present 2 difficulties in yearling stags that are primarily reared for meat production. There are concerns regarding drug residues in meat and antler from spiker deer (Marshall, 1991; Dalefield and Oehme, 1999; Woodbury et al, 2002; Clear & Morris, 2005) and following velvet removal, the antler requires a period of time to heal before transport. If the antler is growing rapidly during this time or there are delays in transport and slaughter the antler may regrow longer than 110mm again requiring a second velvet removal procedure.

The development of a compression method of analgesia for spiker antler removal, The *NaturO*TM ring, by AgResearch provides a solution to these difficulties. It can be put on the pedicle of the deer following the specified method (Anon, 2005). After analgesia is achieved (one hour) the antler can be removed and the deer transported directly to slaughter with the ring still in place. The ring maintains haemostasis and analgesia while the deer are in transit and up until slaughter. There is no time required for healing and there are no concerns about drug residues in either the meat or the antlers.

1.3.2 Effects of the *NaturO*TM ring

Analgesia of the velvet antler?

At the time that the experiments in Chapter 3 of this thesis were conducted, there were few published papers relating directly to the effectiveness of analgesia induced by the *NaturO*TM ring in deer or to the mechanism of proposed analgesia. Since then Nicol et al (2009) have published their work. Matthews et al (1999b) stated that compression is an effective technique for providing pain relief during velvet removal from yearling red deer stags. There was no experimental data presented to substantiate this claim, but reference was made to the interim recommendation for acceptance of the procedure by the National Animal Welfare Advisory Committee (NAWAC).

In the more recent work by Nicol et al (2009) three experiments were done. In the first, electrical stimulation of the antler was applied 4 to 5 times in the first 70 minutes after application of the *NaturO*TM ring by 2 different methods (elastator and cable tied). A peripheral nerve stimulator was used and a “train of four” montage applied at 2Hz. Before application of the ring a mean stimulus intensity of 16mA resulted in an “auriculopalpebral reflex (ear or eyebrow twitch)”. After the ring was applied, the minimum stimulus required to cause the same behavioural response increased to 40mA by 30 minutes and to 55mA by 60 minutes. It is unclear how many stags showed a behavioural response to the electrical stimulation and whether a proportion did not respond at the maximum stimulus of 80mA. In the second experiment comparing lignocaine ring blocks and the *NaturO*TM ring, it was reported that 95% of the stags did not show a head/neck avoidance response after 4 and 30 minutes for the lignocaine ring block and *NaturO*TM rings respectively. In this experiment the same electrical stimulator was used but on different settings (50Hz for 5 seconds and a different outcome behaviour was used. The third experiment compared the two electrical stimulation methods on yearling stags that had *NaturO*TM rings applied. Similar time-threshold curves were generated as in the first two studies for each of the methods. While all stags were reported as not showing a head/neck avoidance response to the maximum stimulus of 80mA using the continuous high frequency stimulus, 37% receiving the train-of-four still responded with the auriculopalpebral reflex at currents of less than 80mA. The authors suggest that the difference between the two electrical stimulation methods was due to leakage of current past the *NaturO*TM ring in the train-of-four method. They do not explain why the continuous stimulation did not also result in leakage of current. Overall, the results suggest that the *NaturO*TM ring causes compression of nerves and impedes their function. The time course of 30 to 60 minutes until apparent loss of sensation is consistent with moderate compression force of myelinated nerves and the development of hypoxaemia affected unmyelinated nerves (Dahlin et al, 1986; Fern and Harrison, 1994b; Rorabeck, 1980; Rydevik and Nordborg, 1980). If myelinated, fast conducting fibres were blocked but unmyelinated C fibres remained functional, it is possible that an acute withdrawal response would not be demonstrated to electrical stimulation or a nick test on the antler. Slow conducting fibres could still be functional and the behavioural response may well be different to the type of sensation experienced by the animal in this case, so while Nicol et al (2009) demonstrated that a withdrawal response was prevented by application of the *NaturO*TM ring for 60 minutes, questions still remain as to whether complete lack of sensation during velvet antler removal was achieved. The experiments in Chapter 3 were conducted at the same time as the work by Nicol et al and this was at the request of Deer Industry New Zealand.

Other effects of the *NaturO*TM ring

Matthews et al (1999b) investigated the effects of *NaturO*TM rings on subsequent antler growth, weight or grading characteristics and found no difference between stags that had been velveted after *NaturO*TM rings versus lignocaine ring blocks. Matthews et al suggested that there were no long term detrimental effects of *NaturO*TM rings or permanent nerve damage. Studies at Invermay (Li et al, 1993; Suttie et al, 1995) found that complete resection of the sensory innervation to the antler did not significantly affect antler development, growth or casting in one year old stags compared to antlers with intact sensory innervation but did reduce antler size. This concurs with previous studies (Suttie and Fennessy, 1985; Suttie et al, 1992) that found resection of the zygomaticotemporal and supraorbital branches of the trigeminal nerve did not affect timing of pedicle or antler development, but did reduce antler size. In the study by Matthews et al, the difference in antler weight at harvest approached significance with those removed using compression analgesia being slightly lighter than antlers removed under lignocaine analgesia (.57 kg vs 0.67 kg respectively). These were relatively light mean two-year-old velvet weights and a larger sample size or the use of deer with greater genetic potential for antler growth may have revealed that *NaturO*TM rings produce similar results to nerve resection.

1.3.3 Compression pain

No research has been published on the potential pain associated with the application of *NaturO*TM rings. In other species, most studies have been conducted on soft tissue which has different anatomy to the antler pedicle of deer.

Rubber rings similar to *NaturO*TM rings are used for castration and tail docking in lambs. Several studies have found that the application of a rubber ring to the scrotum or the tail of lambs results in physiological (Lester et al 1991, Dinniss et al 1997) and behavioural (Molony et al 1993, Kent et al 1995, Grahm et al 1997) responses indicative of pain. Application of the ring to the scrotal neck resulted in increased afferent activity in the spermatic nerves (Cottrell and Molony 1995) and using a smaller ring appears to produce a more intense pain for a shorter period of time (Kent et al 1995). Pain-indicative cortisol and behavioural responses have also been reported in calves castrated with rubber rings (Molony et al 1995).

While there is some evidence that rubber rings applied to young animals are painful, there is very little published data about the effects in adult animals. Tom et al (2002) found that adult cows tail docked with rubber rings showed only mild behavioural changes and no effect on feed consumption or milk yield. This may be a better model for the use of *NaturO*TM rings on the antler pedicle.

There have also been some studies that investigated the effects of tourniquets, which are different from O rings, in animals. Crews et al (1994) found increased activity in the nociceptive-responsive rostral ventromedial medulla in response to tourniquet application on the hind limb of rats for one hour. Tourniquet application to the limb of sheep resulted in reduced threshold to mechanical pain stimulus (Welsh and Nolan 1994, Main et al 1997) and physiological measures in response to

tourniquet application have been recorded in African Grey monkeys (Crews and Sehhorst 1991).

In people, there is sometimes ongoing pain after tourniquet use referred to as chronic post-ischaemic pain and a similar condition may occur in animals. On removal, of a tight O ring tourniquet applied to the distal limb of rats for three hours, there was hyperaemia, swelling and hyperaesthesia both of the limb to which the tourniquet was applied and in the contralateral limb. (Corderre et al 2004). Tourniquet application to the tail of rats results in a co-ordinated escape response and reperfusion after tourniquet release results in a decreased threshold to heat stimulus that increased towards normal levels by NSAIDs (Gelgor et al 1992) and benzodiazepines (Cartmell and Mitchell 1993). It is uncertain whether reperfusion pain would occur in stags after one hour of *NaturO*TM ring application because the antler is removed and there would be very little tissue left to be affected by ischaemic hypoxia. Matthews et al (1999) found no significant effect of the *NaturO*TM rings on the subsequent years antler growth in two-year-old stags, indicating that the blood and nerve supply required for antler regeneration is not permanently damaged by the rings. Reperfusion pain would only be a problem in animals that have the ring removed after velveting, whereas many stags are transported directly to slaughter with the *NaturO*TM rings still in place.

The experiments in Chapter 3 of this thesis use electroencephalography and behaviour to investigate the noxiousness of application of *NaturO*TM rings and the effectiveness of *NaturO*TM rings at preventing a response during velvet antler cutting.

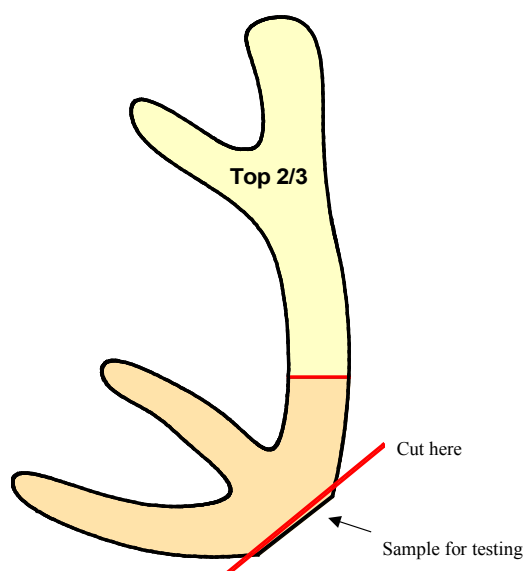


Figure 1.4.4 Sample site for the testing of velvet antler for lignocaine residue levels as indicated by NZFSA. Diagram supplied by S. Morris, NZFSA

1.4 Lignocaine residues in velvet antler

1.4.1 Lignocaine residues and the NZFSA

Lignocaine may be metabolised to a potentially carcinogenic compound, hence the New Zealand Food Safety Authority (NZFSA) has expressed concern at the presence of lignocaine residues in harvested velvet antler.

As at February 2011, velvet antler was the only product for which a maximum permitted residue level (MPRL or MRL) for lignocaine had been specified by the New Zealand Food Safety Authority and this was set at 0.1mg/kg. Table 1.4.1 is copied from Schedule 1 of the New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards 2011, gazetted under the Food Act 1981. According to clause 6(2) (a) and (b) from the gazette, all other saleable edible products are apparently subject to the same value, as 0.1mg/kg is the default MPRL when the product is not specified or an alternative MPRL is not specified.

A 2004/2005 survey by the NZFSA of randomly selected antler sticks found that 50% of antlers tested had lignocaine concentrations at the base of the antler that were above the MPRL (Clear and Morris 2005). Those above the MPRL range from 0.12 to 18 mg/kg. Clear and Morris suggested that compliance with best industry practice for velvet removal should ensure lignocaine levels below the MPRL and cautioned the industry of the possibility of a regulated control scheme if the issue of excessive residues was not resolved. This led to the undertaking of the experiments described in Chapter 4 of this thesis, aimed at providing information on which to base industry best practice for velvet removal and minimising lignocaine residue in velvet antler.

There have been two previous unpublished surveys, two unpublished confidential experiments and one published experiment (Bagonluri et al, 2005) in which lignocaine residues have been quantified in velvet antler. Of these, only the data from Clear and Morris (2005) and Bagonluri (2005) are publicly available.

<i>Table 1.4.1 Copy of an exert from the Schedule one of the New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards 2011</i>				
Column1	Column 2	Column 3	Column 4	Column 5
Compound Name	CAS#	Residue for which the maximum residue limit applies	Food	Maximum Permitted Residue Level (mg/kg)
Lignocaine (lidocaine)	137-58-6	Sum of: Lignocaine 2,6-dimethylaniline Expressed as: 2,6-dimethylaniline	Deer Velvet	0.1

Bagonluri et al (2005) used a sensitive liquid chromatography with UV detection method developed at the University of Saskatchewan, Canada to determine lignocaine concentrations and MEGX (a metabolite of lignocaine) concentrations in elk antlers. Thirteen stags were used (10 treated with lignocaine, and three treated with electroanalgesia acting as controls). Twelve samples were run from each of three sections (base, mid and top) for repeated analyses. Lignocaine residues present in antlers harvested 4 to 6 minutes after administering lignocaine hydrochloride 2% at a dose of 1.2mL/cm pedicle circumference (16-20mL) ranged from 0.068 to 4.3mg/kg. The highest measure was an outlier and none of the other samples were greater than 2mg/kg.

Food safety issues

Lignocaine residues in meat has not been raised as an issue. This may be because lignocaine is rapidly metabolised in the absence of liver pathology (Keenaghan and Boyes, 1972; Boyes et al, 1970; Forrest et al, 1977; Fukada, 2003) so that, by the time the animal reaches slaughter, levels in meat are low. There is no with-holding period specified for lignocaine products registered for use in deer in New Zealand, reflecting the ambiguous international recommendations regarding the use of lignocaine in food animals. The European Agency for the Evaluation of Medicinal Products states that lidocaine (alternative name for lignocaine) may be used in horses for local regional anaesthesia only, and that there was no need to establish an MRL for lidocaine in any species because “it is used in a small number of individual animals only for infrequent treatments, that animals are unlikely to be sent for slaughter during or immediately after treatment and lidocaine is rapidly metabolised and extensively excreted in horses.” The USFDA states that lidocaine should not be used in food producing animals but is contradictory as it also recommends avoiding use immediately before the hunting season. Despite the lack of a with-holding time on New Zealand registered products, the veterinarian administering or prescribing lignocaine must ensure that the MPRL of 0.1mg/kg is not exceeded and applying a withholding period is a sensible approach.

Applying a with-holding period is not possible in the case of velvet antler because the antler must be removed while the drug is acting. There is unlikely to be any further breakdown of lignocaine in the antler after removal as lignocaine is mostly metabolised in the liver by cytochromes CYP1A2 and CYP3A4 and not in other tissues (Nelson et al, 1977). The presence of high lignocaine residues and lack of metabolites in antler some time after harvest supports the proposal that further breakdown does not occur in antlers after harvest (Bagonluri, 2005; Clear and Morris, 2005).

Overdose toxicity of lignocaine in velvet antler

The Xylocaine product data sheet on RxList (<http://www.rxlist.com/xylocaine-drug.htm>) states that the maximum dose for IVRA should not exceed 4mg/kg or 300mg total dose in a healthy adult. The maximum recommended daily dose of velvet in traditional Chinese medicine is up to 9 g/day for 10 days. In the worst case scenario, antler may contain 20 mg/kg of lignocaine (Clear and Morris, 2005). This would result in a total ingested dose of 0.18 mg per day. If the oral bio-availability is assumed to be 100% with no first pass metabolism in the liver, this would be 1/1000 of the dose expected to show some side effects in humans. Alternatively, in order to achieve a dose of 4 mg/kg, a 60 kg adult would need to consume 12 kg of velvet with a residue level of 20 mg/

kg. The rate of oral absorption is likely to be high but due to first pass metabolism in the liver, circulatory concentrations will be low following oral administration (Boyes et al, 1970). The risk of oral lignocaine toxicity in velvet antler is therefore negligible.

Carcinogenicity

There were no reports found of cancer related to lignocaine use in any species.

2,6-xylidine metabolite

Figure 1.4.1 shows the breakdown pathway of lignocaine in humans (redrawn from Nelson, 1977). Nelson et al (1977) quantified 7 metabolites after oral administration of lidocaine to humans including 2,6-xylidine.

The International Agency for Research on Cancer (IARC) has classified 2,6-xylidine (alternative names: 2,6-dimethylaniline or 2,6-DMA or DMHA) as potentially carcinogenic to humans in the 1993 monograph (IARC, 1993). It is this classification that has raised concern over the use of lignocaine in food producing animals. This is in spite of the use of lignocaine, and other local anaesthetics that metabolise to potentially carcinogenic compounds, in human medicine at much higher doses than would be ingested in meat, antler or other animal product. 2,6 xyloidine has to undergo bioactivation before it exerts carcinogenic effects. This involves cytochrome P450 monooxygenation of the amide group to form N(2,6-dimethylphenyl)hydroxylamine (DMHA), followed by esterification. The resultant ester is reactive and readily oxidised to nitrenium ion which can bind DNA and cause cancer (Tyde'n et al, 2004).

Besides being a metabolite of amide local anaesthetics including lignocaine, bupivacaine, etidocaine, mepivacaine and ropivacaine (Carson, 2000), 2,6 xyloidine is found in tobacco smoke (Irvine and Saxby, 1979), as a breakdown product of aniline pesticides, and in the production of dyes, particularly hair dyes.

There is some experimental evidence to show that 2,6-xyloidine may be related to cancers in humans (Gan et al, 2004) and rats (Kornreich and Montgomery, 1990). In a retrospective case control study, human patients that had high levels of blood 2,6 xyloidine-haemoglobin adduct had a higher risk of bladder cancer than patients with low levels of the adduct (Gan et al, 2004). Nelson et al (1977) found that approximately 1% of the ingested dose of lignocaine is excreted in urine as 2,6-xyloidine and Tyde'n et al, (2004) demonstrated that tissue cytochrome P450 is able to convert 2,6-xyloidine to a carcinogenic compounds. These cytochromes may be associated with the bladder wall.

Rats fed diets containing up to 3000 ppm of 2,6 xyloidine for two years had higher incidences of adenomas and carcinomas of the nasal cavity and a higher incidence of rhabdomyosarcoma, subcutaneous fibroma and fibrosarcoma than control rats (Kornreich and Montgomery, 1990). In a study in rats given radioactively labelled ³H-2,6 xyloidine, distribution and binding of 2,6 xyloidine was highest in adipose tissue and tissue binding was most prominent in oesophagus, nasal olfac-

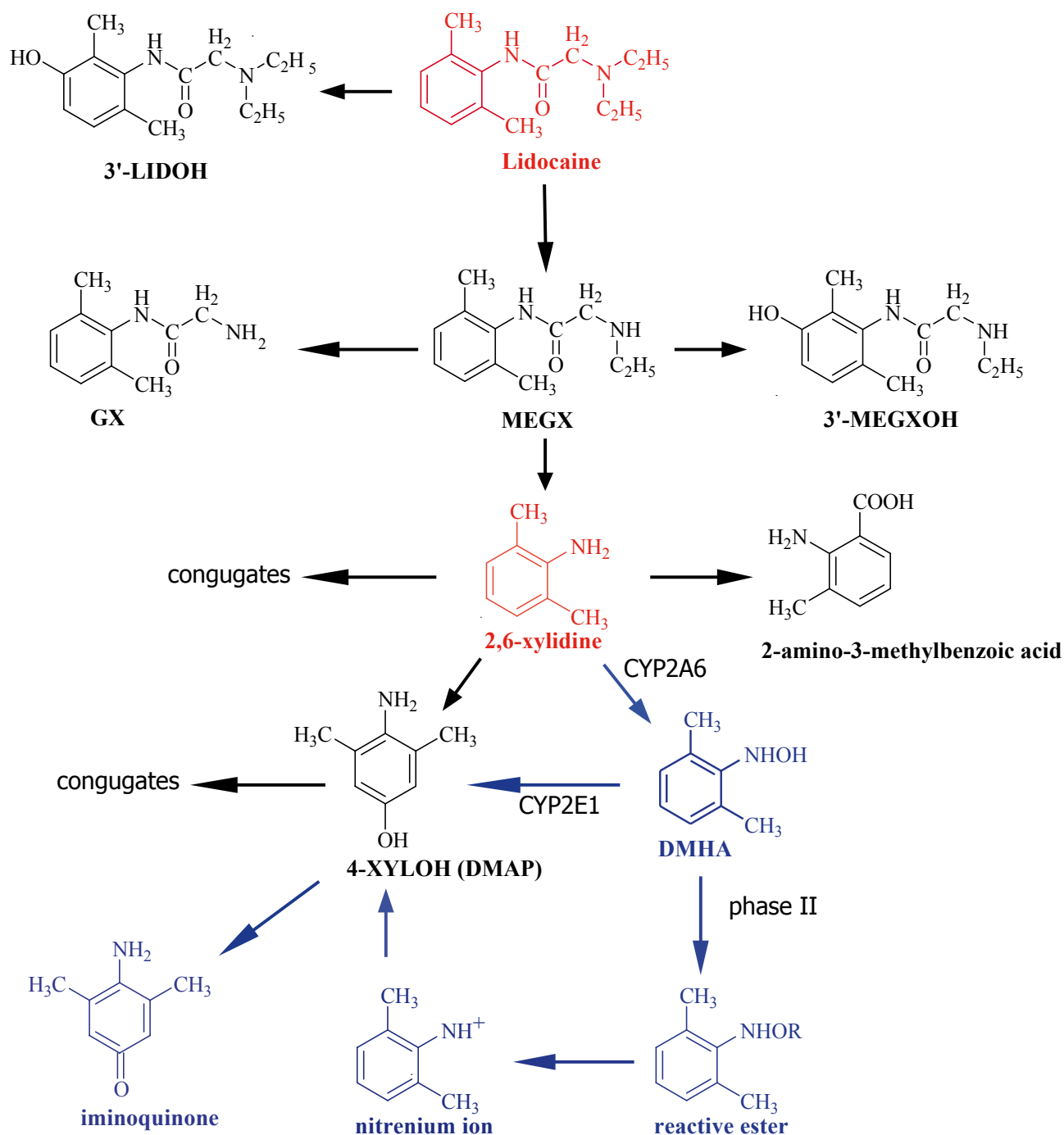


Figure 1.4.1 Breakdown pathway of lidocaine in humans shown in black and proposed activation pathways of 2,6-xylidine to potentially carcinogenic compounds shown in blue. From Nelson et al, 1977

DMHA has the potential to react with haemoglobin and DNA. Nitrenium ion can react with DNA and other macromolecules. Iminoquinone is also a strong electrophile.

tory mucosa and mucosa of the upper respiratory and gastrointestinal tracts (Tyde'n et al, 2004). This study also found that all the tissues studied had the ability to form DNA bound 2,6-xylidine metabolites and that this capacity was greatest in the nasal olfactory mucosa. This may explain the high occurrence of neoplasms in the nasal region of the rats fed 2,6 xyldine.

Tourniquet leakage

Clear and Morris (2005) suggested that compliance with best practice with respect to tourniquet use should prevent noncompliant residue levels in velvet antler. The current recommendation in the Farmers Velvet Antler Removal Programme (Anon, 2005b) is to apply a tourniquet to the pedicle prior to injection of lignocaine below the level of the tourniquet. In the Clear and Morris paper, it was not clear whether this protocol had been followed during the removal of noncompliant velvet antlers. Bagonluri et al (2005) recorded similar levels of lignocaine in antler to Clear and Morris and recent communication with the authors (M. Woodbury, *pers comm.*) confirmed that the local anaesthetic was injected before the tourniquet was placed on these animals.

Even if the recommended protocol is followed, noncompliant residue levels may occur as a consequence of tourniquet leakage and this occurs during intravenous regional anaesthesia in human patients (Machado and Bastos, 1998). Hoffmann et al (1995) measured the amount of a radioactive compound similar to lignocaine that leaked during 20 minutes of tourniquet inflation. In that experiment 29% of the injected volume leaked from the lower limb and 15% from the upper limb. In another study (Quinton et al, 1988), eleven out of sixteen patients undergoing upper limb procedures using a tourniquet and intravenous regional anaesthesia (IVRA) had measurable levels of prilocaine in the arterial circulation during the period of tourniquet inflation.

Bagonluri et al (2005) found no traces of monoethylglycinexylidide (MEGX), a major metabolite of lignocaine, in antlers harvested using lignocaine. This suggests that the lignocaine entering the antler is coming directly from the site of injection and not from circulating blood as the rapid metabolism would mean that some MEGX would be generated on first pass through the liver and should be present in circulation.

It has been proposed that in order to prevent lignocaine entering the velvet antler, blood flow to the antler must be prevented, usually by means of a tourniquet. The tension applied when placing the tourniquet must be great enough to prevent arterial blood flow to the antler, yet not so tight as to be unacceptably painful to the animal. A wide range of tourniquet materials and application methods are used on farms and the results above suggest that these are not always working. The ideal tourniquet has not yet been identified.

The series of experiments in Chapter 4 aimed to investigate the anatomy and dynamics of the blood supply to the antler, the effect of a tourniquet on blood flow and the effect of different tourniquets on lignocaine residue in commercially harvested antler. In addition an attempt was made to develop a standard tourniquet device that might eliminate lignocaine residue in velvet antler.

1.5 Xylazine use and effects in deer

1.5.1 Chemical immobilisation of deer for velvet removal

In order for velvet antlers to be removed safely from deer, the animal must be adequately restrained. Modern, well handled red and wapiti stags can often be safely restrained in a good quality crush especially designed for handling deer. In this situation velvet can be removed under local anaesthesia alone and without the use of chemical tranquilisers. In many circumstances, such facilities are not available or not suitable for handling the animals and chemical sedation is required. Chemical sedation of multiple animals at one time also speeds up the process of commercial velvet harvest making it more economically viable. It is estimated that approximately half of the 500,000 or so stags that are velveted each year are chemically sedated for the procedure (Pearse, pers. comm). As stags are not easily handled and can often be aggressive, in most cases it is not safe for the operator to deliver drugs intravenously, therefore the drugs used must be able to be delivered by the intramuscular route, often with an extended pole syringe and must also be of a small volume that can be injected rapidly as a single bolus. New Zealand farmed red deer have been bred and selected for temperament and ease of handling and can usually be successfully sedated in a pen using a pole syringe or hand held syringe. Other species of deer such as fallow and white-tailed deer tend to be more flighty and easily agitated and darting is often required for immobilisation.

Walsh and Wilson (2002a) summarised the major prerequisites for a safe, effective and efficient immobilising agent as one that is able to be “safely administered by the intramuscular route”, and one that “is easy to administer, provides rapid onset and predictable and repeatable action, provides rapid and uneventful recovery, can be delivered in small volumes, and ideally be reversible. The drug should be compatible with other drugs and be nonirritant. Ideally there should be no drug residues in edible tissue. Above all the agent should be safe for the animal (i.e. have a high therapeutic index), and the operator”.

There are several classes of drugs now available for restraint of deer and these have been studied fairly widely for use in capture of various species of wild deer. These have been reviewed (Haigh and Caulkett, 2004; Walsh and Wilson, 2002a) and, with the exception of xylazine, will only be described briefly here. Overseas, it is more common to use combinations of drugs for capture and immobilisation (Marco and Lavin, 1999; Haigh and Hudson, 1993).

The alpha-2 agonists have a number of properties that make them desirable for use in farmed deer for the procedures that are commonly performed. The level of sedation is dose dependant from a mildly sedated state with the animals remaining standing to heavy sedation and lateral recumbency. Good muscle relaxation is achieved and recoveries are smooth and rapid following intravenous injection of the alpha-2 antagonist, yohimbine (Mackintosh et al, 1984). Xylazine is relatively safe to humans compared to the potent opioids and there is lower risk of misuse of drugs with xylazine than the opioids. This has allowed the drug to be prescribed by veterinarians for administration by

farmers following training and certification specifically for the purpose of velvet removal.

Post-xylazine stag death

Death of stags following sedation with xylazine has concerned veterinarians and the deer industry for many years (Campbell 2001) yet little is known about the mechanism of the syndrome or risk factors that increase the likelihood of deaths. During the 1987/1988 velvet removal season, Walker and Middleburg (1988) described stags dying 12-24 hours after sedation with xylazine in the Hawke's Bay region. Pulmonary oedema and increased numbers of degranulated eosinophils in the pulmonary parenchyma were demonstrated in stags that were postmortemed. These changes were not seen in a follow-up study, described in the same paper (Walker and Middleburg, 1988), examining lung histology of healthy stags one and ten days following sedation, suggesting that xylazine-induced pathological changes occur in some stags but not others. The authors suspected a hypersensitivity reaction, the nature of which has been described by Fletcher (1974). In a subsequent survey in the Hawke's Bay (1988/1989 season) Walker (1989) found a lower incidence of the problem (0.13 per 1000 compared with 1.74 per 1000 the previous season) indicating that deaths may be unpredictable in occurrence from year to year.

1.5.2 Effects of xylazine (and other alpha 2-adrenergic agonists)

The alpha 2 agonist drugs that are currently available for use in veterinary practice include xylazine (Figure 1.5.1), romifidine, detomidine and (dex)medetomidine. Of these only xylazine is commonly used in velveting deer and occasionally combinations of xylazine and ketamine ("Xylaket", Phoenix Pharm distributors Ltd.) or xylazine/fentanyl/azaperone ("Fentaz", Bomac Laboratories Ltd.). A list of xylazine containing products that are registered for use in deer is provided in Table 1.5.1.

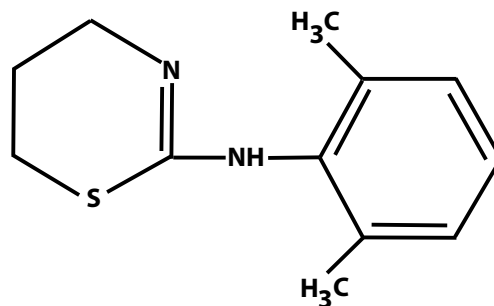


Figure 1.5.1 Chemical structure of xylazine (N-(2,6-Dimethylphenyl)-5,6-dihydro-4H-1,3-thiazin-2-amine).

Action

Adrenergic receptor agonists and antagonists exert their effects on the sympathetic nervous system by either altering or mimicking the native effects of adrenaline and noradrenaline on target cells or altering the release of noradrenaline from nerve terminals.

Alpha adrenergic receptors are G-protein coupled receptors that come in 2 classes, alpha-1 and alpha-2, each with at least 3 or 4 subclasses. Alpha-1 receptors are generally located post-synaptically whereas alpha-2 receptors are found both post and presynaptically. Pre-synaptic receptors

Table 1.5.1 Products registered with the ACVM in 2011 that contain xylazine and are labelled for use in deer

Product (manufacturer)	Active ingredients	Comments
ANASED 2% INJECTION (Lloyd Laboratories NZ Ltd)	Xylazine HCl 20mg/mL	No label documentation available
ANASED 10% INJECTION (Lloyd Laboratories NZ Ltd)	Xylazine HCl 100mg/mL	Main indication is use in horses, also has deer on the label
BOMAZINE 10% (Bomac Laboratories Ltd)	Xylazine HCl 100mg/mL	For sedation of large animals
BOMAZINE 5% (Bomac Laboratories Ltd)	Xylazine HCl 50mg/mL	Horses, cattle and deer. Recommended dose rate for deer 0.8-2mg/kg
XYLAZINE 20MG/ML (Stockguard Labs NZ Ltd)	Xylazine HCl 20mg/mL	Cattle, sheep, horses, deer, goats, dogs and cats.
XYLAZINE 100MG/ML (Stockguard Labs NZ Ltd)	Xylazine HCl 100mg/mL	Cattle, sheep, horses, deer, goats, dogs and cats. Recommended dose rate for deer 0.2-1mg/kg
XYLAZE 100 INJECTION (Bomac Laboratories Ltd, formerly Parnell Laboratories Ltd.)	Xylazine HCl 100mg/mL	Labelled for horses and deer with specific recommendations for deer including Rusa Chital and Fallow. Recommended dose rate up to 1.2mL/40kg in red deer (=3mg/kg) for immobilisation and in combination with ketamine at 2-3mg/kg
XYLAZE 50 INJECTION (Bomac Laboratories Ltd, formerly Parnell Laboratories Ltd.)	Xylazine HCl 50mg/mL	Label contains warning "Use in deer may cause hypersensitivity reactions characterised by respiratory distress and death. Labelled for horses, cattle and deer with specific recommendations for deer including Rusa Chital and Fallow. Recommended dose rate up to 1.2mL/40kg in red deer (=3mg/kg) for immobilisation and in combination with ketamine at 2-3mg/kg
XYLA-SED (Pfizer NZ Ltd)	Xylazine HCl 100mg/mL	Sedative and analgesic for horses and deer. Dose recommendations for Red, Rusa, Fallow, Chital, Mule, Sika, White-tailed, Mule deer and Elk. Recommended dose red deer up to 2mg/kg.
FENTAZIN-5 INJECTION (Bomac Laboratories Ltd, formerly Parnell Ltd.)	Xylazine HCl 58.3mg/mL Azaperone 3.2mg/mL Fentanyl Citrate 0.4mg/mL	Only deer on the label. "A neuroleptanalgesic and skeletal muscle relaxant for use in deer."
FENTAZIN-10 INJECTION (Bomac Laboratories Ltd, formerly Parnell Ltd.)	Xylazine HCl 116.6 mg/mL Azaperone 6.4 mg/mL Fentanyl citrate 0.8 mg/mL	Only deer on the label. "A neuroleptanalgesic and skeletal muscle relaxant for use in deer." Recommended dose for immobilisation of red deer is 0.5mL/100kg.
Xylaze 20 Injection (Bomac Laboratories Ltd).	Xylazine HCl 20mg/mL	Cattle, deer, sheep, dogs and cats.
PHOENIX XYLAKET 15/5 (Phoenix Pharm Distributors Ltd)	150mg/mL xylazine HCl 50mg/mL ketamine HCl	Labelled for use in deer only. "For immobilisation, sedation and analgesia of deer".
PHOENIX XYLAZINE 2% INJECTION (Phoenix Pharm Distributors Ltd)	Xylazine HCl 20mg/mL	Horses deer cattle dogs and cats. Recommended dose for deer 0.6=1.0mg/kg
PHOENIX XYLAZINE 5 % INJECTION (Phoenix Pharm Distributors Ltd)	Xylazine HCl 50mg/mL	Cattle, deer and horses. Recommended dose in deer 0.6 to 1.0mg/kg IM
PHOENIX XYLAZINE 10% INJECTION (Phoenix Pharm Distributors Ltd)		Horses and deer Recommended dose deer 0.6-1.0mg/kg

act as a negative feedback system, inhibiting the release of noradrenaline into the synaptic space or neuromuscular junction. Post synaptic alpha 2 receptor activation has variable effects on different tissues through cAMP cycling and changes in intracellular calcium ions.

Xylazine binds to and activated alpha 2 adrenoreceptors and, to a lesser extent, alpha 1-adrenoceptors may also be stimulated. Other alpha 2 agonists such as detomidine and medetomidine have a higher selective affinity for the alpha 2 receptor than xylazine (Virtanen et al, 1988). Xylazine may also have other direct effects on cells that have not been described. Most of the evidence suggests that the side effects of xylazine can be blocked by selective alpha 2 antagonist drugs and are therefore mediated by the alpha 2 adrenergic receptor on different tissues or the sympathetic nervous system effects on these tissues.

Pharmacology

There is limited data on the pharmacokinetics and pharmacodynamics of xylazine. Xylazine is rapidly absorbed and distributed following intravenous and intramuscular injection. Garcia-Villar et al (1981) administered xylazine via intramuscular and intravenous injection in dogs, horses, cattle and sheep. There was very little difference in absorption, and plasma concentrations over time between the 4 species. Following intravenous administration, the decay curve was biphasic with a short distribution half time of 1.2 to 5.9 minutes, an elimination half time of between 23 and 50 minutes and a volume of distribution of 2 to 2.7l/kg. For sheep, horses and dogs, absorption from the muscles of the neck (horse) or gluteal muscle (sheep, dog) was rapid with a $T_{1/2}$ absorption of 2.7 to 5.5 minutes and peak plasma concentrations occurring at 13 minutes. Bioavailability following intramuscular injection was more variable within and between species and ranged from 17 to 90% across all animals studied (area under the decay curve ratio for intramuscular vs intravenous injection).

Xylazine is likely to be extensively metabolised. Only 8% of the parent radiolabeled compound was recovered from the urine of rats (Duhm et al, 1969) and less than 1% was recovered as parent compound from cattle urine (Putter and Sagner, 1973). Following administration of radiolabeled xylazine to rats (Duhm et al, 1969), approximately 20 different metabolites were detected, but not specifically identified. Some of the metabolites have been characterised in the urine of horses *in vivo* and the same metabolites were isolated from rat liver microsomes incubated with xylazine *in vitro* (Mutlib et al, 1992).

Excretion appears to be mainly via the urine with a smaller percentage in the faeces. Radioactivity was recovered from rat urine to bile at a ratio of 7:3 (Duhm et al, 1969) and from cattle between 76 and 96% of recovered radiolabelled compounds were recovered from the urine with the remainder from the faeces (Murphy and Jacobs, 1975).

Between species dose-response variation

There is considerable between-species variation in the response to xylazine with ruminants generally requiring lower doses for the same level of sedation than non ruminant species (Gross and Tranquilli, 1989). This is likely to be due to the type and number of receptor subtypes in different

tissues. For example the main alpha 2 receptor subtype in the locus coeruleus of the sheep may be the type 2D (Schwartz and Clark 1998a, 1998b) which is not believed to be present in humans. The use and dose differences of xylazine between different species has been extensively reviewed (Greene and Thurmon, 1988). This paper also describes in detail the physiological effects of xylazine in a large number of animal species for which studies had been published at that time.

Variation within deer

Within the family of deer, there is variation in the dose required for equal levels of sedation, although most studies have used combinations with other drugs and most of the published literature concerns white-tailed deer (*Odocoileus virginianus*). Axis deer (*Axis axis*) have been immobilised with up to 3.5mg/kg IM xylazine (Sontakke et al, 2007; Arnemo et al, 1993) without adverse effects noted. In free-ranging moose injected with xylazine, 78% were effectively immobilised at a mean dose of 4mg/kg IM (Delvaux et al, 1999). An intramuscular dose of 0.5mg/kg caused recumbency in all 15 farmed red deer studied by Wilson et al (1996a), whereas doses between 1.5 and 2.0mg/kg IM xylazine were used to immobilise white-tailed deer (Wallingford et al, 1996) but 0.5 mg/kg of xylazine + 4.5mg/kg ketamine was insufficient to adequately sedate white-tailed deer (Van der Eems and Brown, 1986). Roughton (1975) used a range of doses from 0.89 to 8.0mg/kg to immobilise white-tailed deer and found all doses effective with prolonged time of recumbency for doses greater than 3mg/kg but no fatalities up to 8mg/kg. It appears that the effective sedative dose range and safety margin is quite large. There is also likely to be a significant effect of temperament and level of stress in the animals prior to induction, particularly when there are high levels of circulating adrenaline.

Physiological effects of xylazine

Central nervous system

Xylazine causes dose dependent sedation, muscle relaxation and analgesia in all species. The sedative effects of alpha-2 agonists result from suppression of noradrenaline release from the locus coeruleus which is the sole source of noradrenaline to the neocortex, hippocampus, cerebellum and most of the thalamus (Benarroch, 2009). The locus coeruleus noradrenaline system has a major role in arousal, attention and stress response. This has been identified as the site for hypnosis by dexmedetomidine and reversal with prazosin in rats (Correa-Sales et al, 1992).

Analgesia occurs via central, spinal and peripherally mediated actions (Buerkle and Yaksh, 1998; Butterworth and Strichartz, 1993). Analgesia has been described in several species following xylazine administration including horses (Hoffman, 1974; Pippi and Lumb, 1979), rats and mice (Schmitt et al, 1974), and for epidural for caesarian section in cattle (Caulkett et al, 1993; Caron and LeBlanc, 1989). Cutaneous analgesia was related to the level of sedation in red deer following administration of xylazine (Wilson et al, 1996a). The degree of peripheral analgesia provided by xylazine up to 0.75mg/kg IM was considered insufficient for velvet removal in red deer based on a behavioural withdrawal response during antler cutting (Wilson et al, 1996b). In cattle, xylazine alone at a dose rate of 0.248 ± 0.043 mg/kg IV was insufficient to prevent a reaction to skin inci-

sions for fat biopsy (Raptopoulos and Weaver, 1984).

Cardiovascular system

Heart rate

Xylazine causes a decreased heart rate in all species studied by centrally mediated sympathetic inhibition and vagal stimulation (Shirasaka et al, 2007). Decreased heart rate was recorded in red deer (Wilson et al, 1996a) and in axis deer (Armeno et al, 1993) following sedation with xylazine. There are few other reports where xylazine has been used on its own and heart rate recorded in deer. There are considerable data in sheep which have demonstrated decreased heart rate following xylazine (Aziz and Carlyle, 1978; Doherty et al, 1986), detomidine (Clarke et al, 1993; Celly et al, 1997), medetomidine (Ko and McGrath, 1995; Celly et al, 1997) and romfidine (Celly et al, 1997) and in calves following xylazine (Thompson et al, 1989). Xylazine (1mg/kg IV) caused decreased heart rate and sinus arrhythmia in dogs that was reversed by yohimbine (Hsu et al, 1985).

Cardiac arrhythmia

Arrhythmia has been reported following xylazine administration in some species. Several arrhythmias and ECG changes were observed in Mule deer and Mule deer/White-tailed deer hybrids following intramuscular xylazine/carfentanil combination but not medetomidine/ketamine (Caulkett et al, 2000). Arrhythmia due to sinoatrial and atrioventricular block is common in the horse (Wagner et al, 1991; Yamashita et al, 2000). Transient (< 5 minutes) second degree heart block was seen in all 6 sheep injected with 0.15mg/kg intravenous xylazine (Doherty et al, 1986) and Aziz and Carlyle (1978) recorded EEG evidence of second degree AV block and increasing p-qrs interval for 3-5 minutes after injection of xylazine (0.5mg/kg IV) in sheep.

Blood pressure

Effects of xylazine on blood pressure are variable due to centrally mediated and peripherally mediated mechanisms and potential alpha 1 effects of the xylazine or decreased activation of alpha 1 and beta receptors through inhibition of release of noradrenaline across the synapse. Activation of post-synaptic alpha 2 receptors usually causes contraction of vascular smooth muscle and sustained increase in vascular resistance. Decreased blood pressure in spite of increased vascular resistance must be due to decreased cardiac output. Cardiac output, stroke volume and cardiac conductivity were reduced in sheep injected with xylazine and this was accompanied by decreased limb volume and gut volume (Aziz and Carlyle, 1978). This highlighted the combined effects of peripherally mediated vasoconstriction and decreased cardiac output with the net outcome being a decreased in blood pressure following an initial rise. Usually a decrease in blood pressure is seen in calves (Campbell et al, 1979), horses (Wagner et al, 1991) and sheep (Aziz and Carlyle, 1978) but may be preceded by a transient increase. There appears to be a difference between different alpha 2 agonists. Xylazine decreased blood pressure, whereas medetomidine increased blood pressure in calves (Rioja et al, 2008). In dogs, xylazine caused an initial increase in arterial blood pressure followed by a decrease and this was reversed by yohimbine (Hsu et al, 1985). The initial increase in blood pressure may not be seen following intramuscular injection.

Data in deer are sparse. Black fallow deer had increased pulmonary artery pressure (Pearce and Kock, 1989) following anaesthesia with etorphine and xylazine whereas blood pressure was de-

creased in elk following sedation with the same combination of drugs (Paterson et al, 2009).

Haematology

Erythrocytes

Xylazine causes a reduction in venous PCV that is believed to be the result of splenic relaxation. This has been shown in mares (Kullmann, 2011), in camels (Custer et al 1977) and in fallow deer (Stewart and English, 1990). However Aziz and Carlyle (1978) observed significantly increased PCV in sheep following injection of 0.5mg/kg xylazine

Spanish red deer had decreased red cell count, PCV and haemoglobin when they were captured with a xylazine/ketamine combination compared to physically captured deer (Marco and Lavin, 1999). In red deer injected with 0.9mg/kg xylazine showed a significant reduction in haemoglobin, red cell count, packed cell volume and mean corpuscular volume at 0.5 and 1.0 hours after injection but not 6 hours after injection (Mackintosh and Cross, 1989). This decrease was thought to be due to splenic contraction during restraint and splenic relaxation after injection of xylazine and this was confirmed using splenectomised deer (Cross et al, 1989) that did not show similar levels of decrease in red cell counts when injected with xylazine compared to deer with the spleen intact.

Leukocytes

Changes in circulating leukocytes may be of some use in understanding the effects of xylazine in stags and possible mechanism for stag death following sedation, especially if these can be related to inflammatory changes occurring in other tissues, particularly the lungs.

Spanish red deer captured using xylazine and ketamine had a decrease in segmented neutrophil, lymphocyte, monocyte and total leukocyte count but an increased eosinophil count when compared to deer captured by physical means (Marco and Lavin, 1999). Red deer injected with 0.9mg/kg xylazine had decreased basophils after 0.5 hours that returned to normal after 1 hour, decreased lymphocytes at 0.5 and 1 hour, and decreased platelets at 0.5 and 1 hour after injection of xylazine (Mackintosh and Cross 1988). These findings were repeated in 10 red deer hinds (Cross et al 1989) and similar changes were seen by Cross et al (1992). In the latter study, the *in vitro* effects of xylazine on leukocytes was also investigated by inoculating white blood cells with xylazine. Direct cytotoxic effects were observed although it is not possible to say whether these would be seen in vivo because the relative concentration of the drug was higher than what would be expected in circulation of live animals.

Respiratory system

Respiratory rate is decreased, presumably due to central depression, in most species including horses (Bueno et al, 1999) and cattle (Rioja et al, 2008) but tends to be increased in sheep (Doherty et al, 1986; Celly et al, 1997; Hsu et al, 1989). Raptopoulos and Weaver (1984) described respiration in cattle following intravenous xylazine as always more or less laboured but less noisy if the head was extended and Doherty et al (1986) observed a brief period of apnoea followed by

increased respiratory rate in 5 sheep following injection with xylazine.

Oxygenation of blood

The hypoxaemic effects of xylazine and possible mechanism for development of hypoxaemia in different ruminant species have been reviewed by Read (2003).

Hypoxaemia is common in ruminants including sheep (Aziz and Carlyle, 1978; Waterman et al, 1987; Hsu et al, 1989; Celly et al, 1999b), cattle (Raptopoulos and Weaver, 1984), and llama (DuBois et al, 2004), but not horses, dogs or cats. No effect was seen on arterial pH, PaO_2 or PaCO_2 following intravenous xylazine (1.1mg/kg) in dogs (Klide et al, 1975; Haskins et al, 1986) or cats (4.4mg/kg IM) (Haskins et al, 1975). Despite a decrease in respiration rate, Bueno et al (1999) reported that there was no hypoxaemia seen in horses given low doses of medetomidine and xylazine. Similarly, Haskins et al (1986) found no hypoxaemia in horses given clinically sedative doses of xylazine and Wagner et al (1991) found there was a brief decrease in PaO_2 that was not accompanied by an increase in PaCO_2 in horses sedated with xylazine.

There are very few studies using deer in which an alpha-2 agonist has been the sole drug without the addition of an opioid, ketamine or other combination (e.g. zolazepam/tiletamine). Using these combinations, hypoxaemia has been a common finding in many different species of deer including white-tailed deer (Murray et al, 2000), wapiti (Read et al. 2001; Paterson et al, 2009), feral red deer (Janovsky et al, 2000) and Rocky Mountain elk (Kreeger et al, 2011). It is difficult to gauge the level of contribution that the alpha 2 agonist has made to the hypoxaemia, particularly when combined with opioids that are also likely to cause respiratory depression and hypoxaemia.

Read et al, (2001) measured mild to marked hypoxaemia in nine wapiti deer sedated with xylazine (1mg/kg) and tiletamine/zolazepam ("Zoletil") (2mg/kg) given IM into the leg ($\text{PaO}_2 = 43 \pm 11.8$ mmHg) with a slight increase in PaCO_2 . Response to nasal insufflation with oxygen was dramatic with PaO_2 rising to 207 ± 60 mmHg after 5 minutes. These authors recommended nasal oxygen be given to all wapiti deer sedated with xylazine-tiletamine-zolazepam combinations.

Murray and coworkers (2000) recorded the effects of a combination of telazol and xylazine in white tailed deer. Wild female white-tailed deer (n=32) were captured and injected with telazol (2.53 ± 0.16 mg/kg) and xylazine (0.69 ± 0.05 mg/kg) intramuscularly by hand syringe and released until recumbent. A single measurement within 15 to 25 minutes of injection revealed moderate to no hypoxaemia in some of the deer. SpO_2 readings ranged from 70% to 100% and did not appear to be related to the dose of xylazine or other measured parameters. This relative lack of hypoxaemia may be because white-tailed deer lungs are not affected in the same way as wapiti or red deer, or the single measurement taken at some time after a relatively small injected dose of xylazine may not have been sufficient to detect a more marked decrease in oxygen saturation. Hypoxaemia has been reported in white-tailed deer sedated with xylazine and carfentanil and was worsened with the addition of ketamine (Storms et al, 2006).

A transient decrease in SpO_2 that increased after initial immobilisation and levelled to 80 to 95%

was recorded in feral red deer (*Cervus elaphus hippelaphus*) in the Slovak Republic (Janovsky et al, 2000). These deer were darted with intramuscular combinations of either etorphine-acepromazine-xylazine or tiletamine-zolazepam-xylazine. Five Rocky Mountain Elk immobilised with a combination of sufentanil and xylazine demonstrated profound hypoxaemia (PaO_2 means ranged from 24.7 ± 5.8 to 30.4 ± 3.4 mmHg; SaO_2 means ranged from 29 to 48%; SpO_2 means ranged from 38.8 to 49.7%; PaCO_2 means ranged from 54.3 to 61.8 at different time points after immobilisation) indicating respiratory depression was at least in part responsible for the hypoxaemia (Kreeger et al, 2011).

Most of the published experiments on the hypoxaemic effects of xylazine have used sheep. Doherty et al (1986) recorded a marked decrease in PaO_2 and cyanosis from 5 minutes after intravenous injection of 0.15mg/kg xylazine in 6 ewes and Hsu et al (1989) found a significant decrease in PaO_2 and increase in respiratory rate in sheep injected with 0.3mg/kg xylazine. In both of these studies, there was no change in PaCO_2 and the effects were not reversed by yohimbine. Hypoxaemia, along with CNS depression was reversed by tolazoline or idazoxan (Hsu et al 1989). At a higher dose of 0.5mg/kg IV, sheep had decreased PaO_2 and increased PaCO_2 (Aziz and Carlyle, 1978). Sheep given equipotent doses of intravenous xylazine, detomidine, medetomidine and romifidine developed similar levels of hypoxaemia with no difference between treatments (Celly et al, 1997). Body position (recumbency) was not responsible as the sheep were supported in a cradle in an upright position for this experiment and Waterman et al (1987) Waterman et al (1987) observed significant hypoxaemia following very low doses of xylazine (50 $\mu\text{g/kg}$) with sheep remaining standing, that was abolished by administration of idazoxan prior to the xylazine.

Hereford and Friesian cattle given between 0.1 and 0.35mg/kg xylazine IV demonstrated hypoxaemia with arterial oxygen pressures ranging from 34 to 80 mmHg nine to 29 minutes after injection of xylazine (Raptopoulos and Weaver, 1984). This was coupled with an increased PaCO_2 in some instances and a decrease in others. On five occasions animals were sampled more than once and produced similar oxygen tension readings at repeated sampling times. This study also found a significant breed difference in the sedative effects of xylazine. The cattle demonstrated respiratory difficulty described by the authors as inspiratory obstruction which may account for the hypoxaemia, but not the hypocapnia. Cattle were also observed to have weak pulse and pale mucous membranes on initial sedation that improved with time. Rioja et al (2008) showed a significant decrease in PaO_2 and a corresponding significant increase in PaCO_2 in calves following administration of intravenous xylazine or medetomidine (12 calves per treatment) which was rapidly reversed by atipamezole.

The mechanism for the hypoxaemia seen in ruminants is still unclear. It appears to be mediated by peripheral α_2 adrenoreceptors and this was demonstrated by Eisenach (1988). In that study, sheep were injected with either clonidine or ST-91. Both of these are α_2 adrenergic agonists but ST-91 does not cross the blood-brain barrier. Both induced hypoxaemia in the sheep although this was more severe with the clonidine. Clonidine induced hypoxaemia was inhibited by the α_2 antagonists, idazoxan and also by the hydrophilic α_2 antagonist DG-5128 but not to the same extent as idazoxan. The α_1 antagonist, prazosin did not prevent hypoxaemia induced by clonidine. This demonstrates that peripheral α_2 receptors are largely but not wholly responsible for the hypoxaemia and that α_1 receptors are not responsible. Hypoxaemia is not

prevented by pre-treatment with aspirin (Nolan et al, 1990), antihistamines (Nolan et al, 1986) or nalaxone (Eisenach, 1988).

Airway pressure

Xylazine and clonidine cause increased airway pressure in anaesthetised, ventilated sheep that was inhibited by the alpha 2 antagonist, idazoxan, but not by the alpha 1 antagonist, prazosin or by antihistamine. The increase in airway pressure was not demonstrated when xylazine or clonidine were injected into the cisterna magna (Nolan et al, 1986). Celly et al (1999b) measured significant changes in PaO₂, pulmonary resistance and transpulmonary pressure following very low doses of medetomidine (0.5 µg/kg) administered to anaesthetised, ventilated sheep. There were no corresponding vascular changes and no change in pulmonary artery pressure in that study.

The alpha 2 agonists, clonidine and medetomidine decreased bovine tracheal smooth muscle contractile tension in response to electrical stimulation, but not exogenous acetylcholine (Manning et al, 1995). These effects were eliminated by addition of alpha 2 antagonist, indicating that alpha 2 adrenoceptors in the bovine trachea cause decreased contractility. This may increase airway pressure and reduce inspired oxygen.

Pulmonary Arterial Pressure

Increased vascular resistance in the lungs would be expected to increase pulmonary artery pressure. This may be induced by vascular smooth muscle contraction or increased resistance in the lungs, for example due to pulmonary oedema. The proportion of shunting of unoxygenated blood would be greater in this case resulting in hypoxaemia.

Despite decreased systemic blood pressure, the pressure within the pulmonary artery is often increased. Blood pressure in the pulmonary artery was increased in calves following intravenous medetomidine and xylazine (Rioja et al, 2008). Black fallow deer had increased pulmonary artery pressure following anaesthesia with etorphine and xylazine (Pearce and Kock, 1989).

Medetomidine at very low doses (0.5 µg/kg IV) causes hypoxaemia in sheep by directly altering pulmonary function without increasing cardiac function or pulmonary arterial blood pressure (Celly et al, 1999b). In another study (Eisenach, 1988) both clonidine and ST-91 caused hypoxaemia in sheep. Clonidine caused a greater decrease in arterial oxygen levels than ST-91. ST-91 caused increased mean pulmonary arterial pressure whereas clonidine did not. These results suggest that increased vascular resistance in the lungs is not wholly responsible for hypoxaemia.

Histological changes

Perhaps one of the most revealing studies was histological examinations of the lungs of sheep at various times after injection of xylazine and ST-91 (Celly et al, 1999a). Severe pulmonary changes included extensive damage to endothelium, damage to alveolar type 1 cells and pulmonary intra-vascular macrophages (Rogers et al, 1994), intra-alveolar haemorrhage and interstitial and alveolar oedema. This was evident from 3 minutes until 60 minutes after injection of xylazine (150 µg/kg) and 3 minutes after ST-91 (30 µg/kg). Lung damage was reversed by 12 hours post xylazine injection. Marked histological changes were also observed by Bacon et al (1998) in sheep lungs after administration of xylazine (200 µg/kg). These included widespread alveolar capillary con-

gestion, intra-alveolar oedema that was often eosinophilic and extravasated red blood cells. The findings of pulmonary oedema and haemorrhage are similar to postmortem findings described by Walker and Middleberg (1988) in stags that had died after xylazine administration although in that report there was marked infiltration of eosinophils which was not described by Celly et al (1999a) or Bacon et al (1998) in sheep. Walker and Middleberg did not mention damage to pulmonary intravascular macrophages (PIMs) or make mention of these macrophages. This type of cell has been identified in deer (Carrasco et al, 1996) and found to be twice as numerous as, and smaller than, alveolar macrophages.

Bacon et al (1998) attempted to identify whether the histological changes were induced by free radical damage from an inflammatory release from activated pulmonary intravascular macrophages. Using a method involving ascorbyl radicals and PBN, these researchers determined that there was no increase in free radicals and that an inflammatory mediated cellular response was not responsible for the pulmonary damage. The most likely mechanism was an alpha 2 adrenoceptor mediated intense venospasm immediately after injection of xylazine that resulted in congestion and extravasation of red blood cells and pulmonary oedema. This is supported by the reversal of hypoxaemia after administration of alpha 2 antagonist but does not account for the changes in PIMs described by Celly et al (1999a).

Other side effects of xylazine

Xylazine has variable effects on many other body systems. Depressed appetite seen following administration of xylazine to red deer calves (Simpson et al, 1993) and white-tailed deer (Warren et al, 1984) may result in reduced growth following sedation. The effects of xylazine on appetite were not reversed by caffeine or yohimbine (Van der Eems and Brown, 1986) and may be related to reduced gastrointestinal motility. This occurs in calves (Guard and Schwark, 1984) and dogs (McNeel and Hsu, 1984) and may also be seen in deer.

An important consideration when sedating deer with xylazine is the potential for pathological changes in body temperature. In a study comparing a combination of xylazine/tiletamine/zolazepam with xylazine/etorphine/acepromazine, 3 out of 60 feral red deer died during immobilisation and this was considered to be due to hyperthermia (Javanosky et al, 2000). The hyperthermia may have been induced by a prolonged chase period and the reduced ability to thermoregulate following administration of the drug may have contributed to the deaths. Alterations on body temperature in cattle following administration of xylazine are affected by ambient temperatures (Fayed et al, 1989) and may also be related to the age and surface area to volume ratio as hypothermia has been reported in foals (Robertson et al, 1990). There is little information about the effects of xylazine on thermoregulation in deer or the critical ambient temperatures for maintaining thermal homeostasis.

The effects of alpha 2-agonists on glucose metabolism have been well described. Hyperglycaemia has been demonstrated in horses (Greene et al, 1987; Thurmon et al, 1984; Tranquilli et al, 1984), dogs (Hsu, 1988), cattle (Eicher et al, 1979; Fayed et al, 1989; Gorewit, 1980; Symonds and Malinson, 1978), sheep (Brockman, 1981; Muggaberg and Brockman, 1982), camels (Custer et al, 1977) and deer (DelGuidice et al, 1988; Janovsky et al, 2000; Mautz et al, 1980). The reason for

the hyperglycaemia may be due to decreased insulin as this had been a common finding in most reports. It is probable that alpha 2-adrenergic receptors in the islet beta cells of the pancreas inhibit the release of insulin resulting in the hypoglycaemia reported. Most of the studies above have shown reduced blood insulin levels and Greene et al (1987) and Hsu (1988) demonstrated reversal of hyperglycaemia by the administration of yohimbine.

Alternative sedative drugs

Opioids

Etorphine, carfentanil, thiafentanil and fentanyl are potent opioids that have been used alone and more commonly, in combination with other drugs. Etorphine, a highly potent synthetic opioid was developed in 1960 and large animal Immobilon (Etorphine + Acepromazine) was developed by MacFarlan-Smith and Co in 1963 and is now manufactured by Novartis Animal Health, UK. It is a popular drug for immobilisation of large game animals such as elephants because of its high potency and rapid onset and because it is fully reversible with the opioid antagonist, diprenorphine. Immobilon was used experimentally in deer in New Zealand (Mackintosh, 1985) but never became available commercially in this country. Deliberate (Sterken et al, 2004) and accidental (Firn, 1973; Goodrich, 1977; Orr, 1977) fatal and non fatal self injection has occurred rarely in humans. Fentanyl is available in New Zealand. Originally this was formulated in combination with azaperone ("Fentaz") and now is formulated in combination with both azaperone and xylazine ("Fentazin" Bomac laboratories Ltd.). Opioids are commonly used in combination with other drugs, especially alpha-2 adrenergic agonists largely to help overcome the side effects of excitement and extrapyramidal effects induced by the opioid during induction.

Phenothiazines and Butyrophenones

These post ganglionic dopamine receptor blockers (Janssen, 1965) have limited use for velvet removal largely due to their slow onset and insufficient degree of immobilisation to safely remove velvet antlers when used alone. Acetylpromazine is incorporated into "large animal immobilon" with etorphine as a sedative to help overcome the excitatory effects of the opioid. The long acting phenothiazine, zuclopenthixol has been used in wapiti deer in Canada to overcome background flight behaviour when assessing the efficacy of analgesia during velvet removal (Read et al, 2000; Woodbury et al, 2001; Woodbury et al, 2002) and to reduce stress during relocation of white-tailed deer (Read and McCorkell, 2002). The slow onset, very long action and irreversibility of these drugs make them less suitable for velvet removal.

The butyrophenone, azaperone is incorporated with fentanyl and xylazine in the combination drug "Fentazin" which is used in deer in New Zealand, although less commonly for velvet removal than xylazine alone. Note that the reversal agent for "Fentazin" is "Contran H" which contains yohimbine and naloxone so that the opioid and the xylazine are reversed but not the azaperone.

Benzodiazepines

Benzodiazepines potentiate the inhibitory effects of gamma-aminobutyric acid and have anxiolytic effects. They are used in combination anaesthetic protocols in other species but rarely used alone in deer. Oral diazepam has been used to facilitate capture of pronghorn (Pusateri et al, 1982) and white-tailed deer, mule deer, axis deer, sika deer, fallow deer and sambar deer (Thomas et al, 1967). When diazepam is administered alone, deer may become more aggressive due to the anxiolytic effects. Zolazepam is sometimes used in the combination drug “Zoletil” (Virbac NZ Ltd,) or “Telazol” (Fort Dodge Animal Health) where it is formulated in combination with tiletamine. There are a number of published reports on the use of these drugs in various combinations with opioids and alpha 2-adrenergic agonists from overseas e.g. in fallow deer (Fernandández-Morán et al, 2000) red deer (Janovsky et al, 2000; Janicki et al, 2006), white-tailed deer (Miller et al, 2003; Murray et al, 2000) Rocky Mountain elk (Millspaugh et al, 1995).

Cyclohexamines

Cyclohexamines such as ketamine and tiletamine are NMDA receptor antagonists that produce analgesia and unconsciousness. They have been used in deer and are reviewed by Haigh and Caulkett (2004). The major limitation with these drugs is poor recovery and increased risk of post sedation capture myopathy. This can largely be overcome by the addition of a benzodiazepine such as zolazepam and/or an alpha-2 agonist. “Xylaket” is an example of such a combination that is available for use in deer in New Zealand and is a combination of xylazine and ketamine. Combinations of tiletamine and zolazepam are used overseas as described above.

Neuromuscular blocking agents

Succinylcholine, while causing rapid immobility and reversal of immobilisation compared to etorphine and xylazine (McCorquodale et al, 1988), does not produce CNS depression. The animal remains affected by visual, auditory and tactile stimuli (Parker and Haigh, 1982) and presumably stress and is neither safe nor humane. Moose immobilised with succinylcholine died of respiratory paralysis during sedation (Delvaux et al, 1999). There are no products containing succinylcholine that are registered on the ACVM list in New Zealand.

Summary

Lignocaine hydrochloride has been shown to provide analgesia for velvet antler removal with variable success rates between operators. There is a need to determine which factors make some operators better than others at administering local anaesthetic. Chapter 2 of this thesis aims to identify these factors so that best practice for administering lignocaine for velvet antler removal can be better defined. In order to determine the effectiveness of the analgesic, the behaviours associated with velvet antler pain require investigation and validation. This is also studied in Chapter 2.

Compression analgesia using *NaturO*TM rings offers several advantages to the deer industry yet the efficacy and potential noxiousness of the *NaturO*TM rings has not yet been determined. The experiments in Chapter 3 of this thesis use electroencephalography and behaviours to answer these questions.

Lignocaine residues in velvet antler have been identified as a food safety issue. It is hypothesised that a suitable tourniquet system can prevent noncompliant lignocaine residues in velvet antler. In order to develop such a system, a better understanding of the anatomy and dynamics of the arterial supply to the antler is required. Chapter 4 describes the use of fluorescent microspheres, cineangiography, acrylic casting and tissue lignocaine analysis to determine the effects of tourniquets on antler blood flow and antler lignocaine residues.

Post-xylazine stag death is a phenomenon that requires further understanding both in terms of its epizology and exact mechanism so that steps can be taken to reduce the incidence of this condition. In Chapter 5 a survey approach was used to collect data relating to post xylazine stag deaths and clinical observations of the hypoxaemia induced by xylazine were made.

Chapter 2

Lignocaine ring block for velvet analgesia

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The effect of ring block method on behaviour during velvet antler cutting in adult stags

Introduction

For an full introduction to this chapter please refer to Chapter 1.2

Velvet antlers are harvested from stags when they are growing and have a well developed neurological (Wislocki and Singer, 1946; Vacek, 1955; Adams, 1979; Woodbury and Haigh, 1996) and vascular (Waldo et al, 1949; Adams, 1979; Suttie et al, 1985) supply. Velvet antlers are sensitive to pain as shown by a behavioural response to electrical stimulation of the antler that is abolished by the application of local anaesthetic around the base of the antler (Matthews et al, 1992; Wilson et al, 1999, 2000; Woodbury et al, 2001, 2002) and an electroencephalographic response in deer under general anaesthesia when the velvet antler is cut without local analgesia (Johnson et al, 2005). Under New Zealand law, velvet antler removal is classified as a controlled surgical procedure and requires analgesia (Animal Welfare Act, 1999). Lignocaine hydrochloride (lidocaine (Gordh, 1949)) administered subcutaneously in a ring block is the most widely used method of providing analgesia. The efficacy of analgesia achieved using this method depends on the correct placement of the drug in relation to the nerves supplying the antler. While good efficacy has been achieved under experimental conditions (Wilson et al, 1999, 2000), variation in the analgesic success is seen when different operators (farmers and veterinarians) have been observed in the field (Morrow and Matthews, 1998; Webster et al, 2003). Between operator variation in analgesic success of 16.7% to 90% was reported by these researchers and this may be due to lignocaine injection technique. For the purposes of quality assurance it is important that operators are trained in the most reliable, repeatable and reproducible methods. Local anaesthetic dose rate and placement site appear to be important factors for achieving analgesic success (Wilson et al, 1999, Bartels et al, 2001). The aim of this research was to examine in detail the factors that contribute to the success or failure of analgesia to the velvet antler when adult stags on commercial deer farms are velveted by farmers and practicing veterinarians so that best practice can be better defined and the industry have improved tools for ensuring the welfare of stags velveted by farmers and veterinarians on commercial farms.

In order to determine whether an analgesic is effective, the absence of a pain response must be identified. Behaviour is the most appropriate method in this situation but the exact behavioural responses that will best identify failure of analgesia have not been well defined. Methods used in previous studies (Matthews et al, 1992; Wilson et al, 1999; Webster et al, 2002, 2003) have involved grading the movement based on the amount of the animal's body that was involved. No published studies were found that validate this method or determine the sensitivity and specificity for detecting failure of analgesia. This study aimed to identify behaviours that were more sensitive and specific indicators of a local analgesia failure during antler removal.

A non-intervention video observational study with retrospective analysis was used to investigate these questions.

Materials and Methods

Animals

Red deer and red crossed with wapiti stags 2-years and older (n=228, 455 individual antlers) were observed undergoing normal velveting procedures on the farms. For all stags this involved physical restraint in a suitable crush, followed by administration of subcutaneous lignocaine and removal of the antlers with a saw.

Operators and properties

Table 2.1 lists the locations of the 16 properties (A to P) on which the observations were made. Nineteen commercial deer farmers and four veterinarians volunteered (numbered 1 to 23). One operator took part on three of the properties and four of the properties hosted two or more operators. The identity of the operators was confidential, and farmers and veterinarians participating were given a written assurance of anonymity. This research project targeted the analgesic block technique independent of the operator and was not part of any auditing or operator assessment programme. All velveting equipment including tourniquets, 2% lignocaine and saws was provided by the operator.

On Farm Recordings

All operators were given a copy of the study protocol and understood the objectives of the study. Throughout, it was important that operators were able to practise their normal velveting procedure. All equipment used for the velveting process was supplied by the operator or farm on which observations were taking place. On 12 of the 16 properties, one researcher only was present. On the remaining four properties, two researchers were present. Equipment was set up in such a way as to provide minimal disturbance to the animals or delays to the rate of the procedure.

Figure 2.1 shows set-up from still images captured from video recordings. A Sony Handycam digital video camera recorder (DCR-PC109E) with a wide angle lens was mounted onto a small flexible tripod attached to a wooden board and screwed above the crush so as to view the whole crush and animal. This camera was run continuously throughout the observations and recordings designated as “W” during placement of local anaesthetic and removal of velvet. The camera was operated with a long-life (5 hour capacity) battery.

A second, held-held, Sony Handycam digital video camera recorder was operated throughout the local anaesthetic application and velvet removal periods. These recordings were designated as close up (“C”).

A recording sheet was completed for each stag. A copy of this is shown in Figure 2.2. These sheets were made into pads of self-carboning copies with 50 sheets per pad.

Table 2.1 Farm ID, location, operator and number of stags per operator for on-farm observations of local anaesthetic technique and behaviour outcome during velvetting.

Location	Farm	Operator	Number of stags
Manawatu	A	1	5
		2	5
		3	5
		5	9
		6	9
Hawkes Bay	B	4	10
Manawatu	C	7	6
Canterbury	D	8	10
	D	9	12
	D	2	10
	D	11	3
	E	10	9
	E	2	9
Marlborough	F	12	12
West Coast	G	13	7
Marlborough	H	14	11
Otago	I	15	9
Southland	J	16	10
	K	17	8
	L	18	10
West Coast	M	19	11
South Otago	N	20	15
	O	21	7
South Canterbury	P	22	23
	P	23	3
Total	16	23	228



2.1 a



2.1 b



2.1 c



2.1 d



2.1 e

Figure 2.1 Setup of cameras for filming analgesic application and efficacy. a) View from the wide-angle video recorder showing the author holding the hand-held video recorder filming close up of the farmer administering the local anaesthetic. b) View from close-up video c) Author measuring the “pedicle circumference” to determine “dose” per cm of pedicle d) Close up view of pedicle measurement of 12cm. e) Author writing details onto the recording sheet

Observation Number _____ Operator Number _____ Farm ID _____		Pedicle circumference _____ mm Beam length above trez _____ mm		Stag ID _____ Age _____ Weight _____	

Time	Action	Response	Comments
	In crush area	Standing quiet ☐ Head and neck, steps ☐ Agitated kicks, grinds ☐ Whole body struggle ☐	
	Crush engaged	☐ ☐ ☐ ☐	
	Tourniquet application	☐ ☐ ☐ ☐	
	1st block L R (circle)	☐ ☐ ☐ ☐	
	2nd block L R (circle)	☐ ☐ ☐ ☐	
	Waiting period	☐ ☐ ☐ ☐	
		No movement ☐ 0 Slight head, eye or facial muscle ☐ 1 Mod. avoidance head shake ☐ 2 Whole body struggle ☐ 3	
	Nick test first antler L R	☐ ☐ ☐ ☐	
	First antler cutting L R Start	☐ ☐ ☐ ☐	
	Middle	☐ ☐ ☐ ☐	
	End	☐ ☐ ☐ ☐	
	Nick test 2nd antler L R	☐ ☐ ☐ ☐	
	Second antler cutting L R Start	☐ ☐ ☐ ☐	
	Middle	☐ ☐ ☐ ☐	
	End	☐ ☐ ☐ ☐	
	Released from crush	Walks out quietly ☐ Requires push ☐ Rushes out—agitated ☐	
	Tourniquet released	Standing quiet ☐ Head and neck, steps ☐ Agitated kicks, grinds teeth ☐ Whole body struggle ☐	

Diagram 1 – top view

Diagram 2 angle view

Indicate location of injection and order (1, 2, 3, 4 etc.)

Rostral

Left Right

Right Left

TISSUE IMPEDENCE
 0= very easy. 3= can't inject

LA Administration Description

Volume LA (total) _____ # injections left _____ right _____ LA Batch number _____

% of circumference blocked _____ (see diagram) Type or syringe _____ Needle size/type _____

Describe injection technique (needle straight/angled, inject as withdrawing, redirected needle)

Tourniquet type and application _____

Figure 2.2 Copy of the original recording sheet used for recording details for local anaesthetic application and efficacy study. Note that only some of these details (indicated in bold) were recorded on this sheet as the rest of the information was entered directly onto excel spreadsheet from video recordings at a later time.

Video viewing

Table 2.3 shows the order of observations made from the video viewing. Wide angle and close up tapes were observed together on adjacent monitors and each set of video recordings was watched twice. On the first occasion, the times for all events were recorded (see below) and all “movements” made by the stag from tourniquet application to cutting of the second velvet antler were recorded. A judgement of whether or not a withdrawal response was demonstrated during antler cutting was also made on this first viewing. During the second viewing, details of the technique used to inject lignocaine around the base of each antler were recorded. Note that each antler was treated independently with respect to the timings, withdrawal response and lignocaine injection technique.

Times derived from the video recorders timing system were recorded for the following:

- Start and finish tourniquet
- Start and finish ring block application antler 1 and 2
- Nick test antler 1 and 2
- Start and finish cutting of antler 1 and 2.

Recording “behaviours” “grades” and “movements”

For the purposes of this study the following definitions of “behaviour” “grade” and “movement” were used:

A “**behaviour**” was defined as an individual action that could be classified and described and was not broken into smaller components. More than one “behaviour” could occur at a time, e.g. “Flinch” (Table 2.6).

A “**grade**” was a type of “behaviour” that described the proportion of the head and body that was involved. “Grades” were mutually exclusive. A “movement” could only include one “grade” (Table 2.5).

A “**movement**” was an action demonstrated by the animal that had a definite start and finish and included one or more “behaviours”, e.g. 2 fl HA was a “movement” that consisted of a “grade” 2 “behaviour”, a flinch “behaviour” and a head arch “behaviour” at the same time. All “movements” must have had a “grade”.

Deciding which “behaviours” to record: Initial video viewing notes

Table 2.2 shows the behaviours and comments that were noted during the initial video viewing.

Those that are listed as “potential withdrawal response indicators” were observed during a potentially noxious stimulus (pulling on the tourniquet, injecting local anaesthetic, cutting the antler) and those listed as “Non-specific behaviours” were usually observed at times when no potentially noxious stimulus was being applied. There was cross-over between these categories.

Videos were analysed by a single experienced researcher to maintain consistency in categorising descriptive data. Before analysis, discussion and preliminary viewing of several hours of recordings were undertaken with three animal behaviour researchers, a neurophysiologist and a statistician to determine which variables would be measured and how measurements would be determined for each.

Tables 2.5 and 2.6 show the actual “behaviours” that were used in the scoring system. The decision to include these particular behaviours and not others from the list in Table 2.2 was based on frequency of use, perceived sensitivity and specificity and ease of visualisation from the video tapes. There were 4 “grades” of “behaviour” and 4 specific head related “behaviours” used in the final analysis.

Recording “movements”

After identifying the “behaviours” that were to be recorded, each animal was observed on both wide angle and close up videos simultaneously and each individual “movement” was scored according to the flow chart shown in Figure 2.3. For each “movement”, the time that it occurred and the part of the velvet removal procedure being performed was recorded. If a potentially noxious

Table 2.2 Behaviours observed during initial video observation of stags during velveting when a potentially painful stimulus was and was not being applied

Potential withdrawal response Indicators	Non-specific behaviours
• Sharp reaction • Same time as cutting • Repeatable • Flinch • Pull away • Tilt head away from stimulus • Arch neck • Eyes widen • Head sink head tilt • Nod head nose down • Throw head backwards • Eye flicker (beware visual stimulus) • Bulging eye • Gland flaring changes after or during cutting • Whole body struggle • Brace neck and point both ears forward	• Blowing • Licking lips • Growl • Gland flaring • Frothing at the mouth • Kick • Jump • Stamp • Whole body struggle • Head and neck • Head only

Table 2.3 Order of observations for recording behaviour outcome and injection technique to determine factors that contribute to the likelihood of a withdrawal response during antler cutting in adult stags on commercial farms

First Viewing	Times (tourniquet, injection, cutting) Movements Withdrawal response assessment
<i>Separation in time of at least 3 hours and in space by 5 animals</i>	
Second viewing	Injection technique Haemorrhage

Table 2.4 Criteria for subjective assessment of withdrawal reaction to the nick test or antler cutting of adult stags velveted by farmers and veterinarians on farms.

Movement must occur simultaneously with potentially painful saw pressure
Repeated movements of any grade while cutting in the same antler region
Increasing intensity of movement with each saw cut
Flinch (see Table 2.6)
Head arch (Table 2.6), particularly associated with small movements or flinch
Operator stops and either allows more time or injects more local anaesthetic
Significant movements occur during cutting, but not other times in the procedure for that animal
Movement of the antler away from stimulus (head down or head rotate)(Table 2.6)

Table 2.5 Description of “grade” “behaviours” assigned to “movements” during application of tourniquet through to cutting of the second antler of adult stags.

Grade	Description
Slight	Movement of the head only in any direction by a distance less than the width of the nose of the stag. Includes facial muscle tensioning.
1	Movement of the head only in any plane
2	Movement of the head and neck to the level of the shoulders
3	Whole body struggle
Looking around (Look)	Slow movement involving orientation of head, eyes and ears to outside stimulus.

Table 2.6 Description of specific “behaviours” recorded during application of tourniquet through to cutting of the second antler in adult stags on commercial farms.

Behaviour	Description
Flinch	Abrupt small movement involving sudden tensioning of the muscles of the head or neck. This may be immediately followed by a grade 1, 2 or 3 body movement, HD, HA or HR
Head arch (HA)	Dorsal displacement of the poll and caudal displacement of the nose
Head down (HD)	Ventral displacement of the whole head towards the restraint
Head rotate (HR)	Rotation around a nasal-caudal axis with ventral displacement of one antler and dorsal displacement of the other

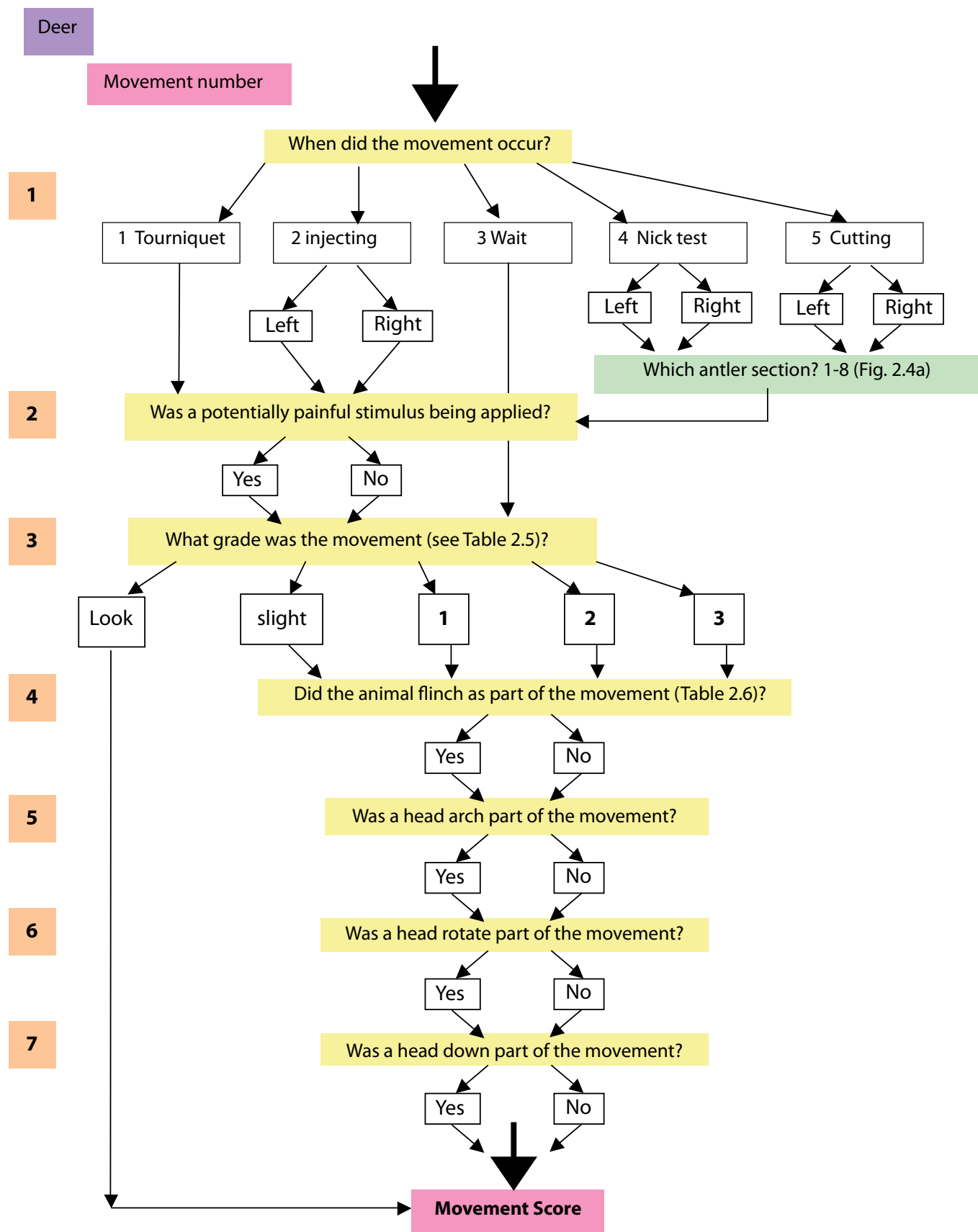


Figure 2.3 Flow diagram for categorisation of each movement for an animal during restraint and manipulation for velvet antler removal.

stimulus was being applied this was recorded as an x. The “movement” always included a grading according to the amount of the body involved (slight, 1, 2 or 3), and could include any of the four specific “behaviours” (flinch, head arch, head down or head rotate). For “movements” that occurred during antler cutting the “Region” of the antler being cut as shown in Figure 2.4a, was also recorded in parenthesis. If a potentially noxious stimulus was being applied it was recorded with an “x”. If no potentially noxious stimulus was being applied a “o” was used. A full list of individual “movements” for every animal is shown in Appendix 2.1 attached as an excel spreadsheet on compact disk.

Statistical analysis of “behaviours”

Specific “behaviours” were analysed statistically to determine whether they occurred more often when a potentially painful stimulus was applied, when the velvet antler was being cut or at other times when the animal was not being handled. Potentially painful stimuli included pulling the tourniquet tight, injecting lignocaine and nick testing or cutting the antler but did not include holding the antler with the hand or restraining the deer in the crush. This was to help determine the relevance of these “behaviours” as potential indicators of a withdrawal response due to pain. The proportion of “movements” that included each “behaviour” was calculated for different stages in the procedure and a Z-test for difference in proportions was conducted to compare the occurrence of different “behaviours” during potentially painful stimuli, during antler cutting when a withdrawal response did not occur, during antler cutting when a withdrawal response did occur, and during other times when the animal was not being directly handled. Basic retrospective diagnostic test parameters were calculated manually without the use of statistical software as the equations were simple. These included sensitivity, specificity, positive predictive value, negative predictive value, positive diagnostic likelihood ratio, negative diagnostic likelihood ratio and non-specificity.

Withdrawal response

Based on the results of the “movement” and “behaviour” scoring, for each antler, a binomial outcome of either a withdrawal response or no withdrawal response during antler removal was determined. For stags that demonstrated a withdrawal response during antler removal, the analgesic was deemed to be insufficient. A subjective assessment was made initially based on the criteria presented in Table 2.4. Two or more of these criteria were required to constitute a withdrawal response. In all cases a withdrawal response was only recorded if the reaction occurred simultaneously with direct potential painful saw pressure. Reactions were recorded as yes (withdrawal response), no (no withdrawal response) or equivocal when it was not possible to determine a response subjectively. Responses were not graded according to severity. Based on the analysis described above of “behaviours” and determination of suitability for deciding a withdrawal response, equivocal case data were revisited without reviewing the video tapes, and a withdrawal response outcome of “yes” or “no” assigned based on the recorded data. This achieved a complete outcome

data set with no remaining equivocal cases.

Independent variables

Table 2.7 lists and describes the independent variables studied. Some of these variables were determined before the start of the study and others, particularly those relating to the actual injection method, were determined after watching the videos several times. It was not known what these would be before the start of the study and flexibility was allowed to account for this. The recording sheet shown in Figure 2.2 was completed including diagrams of local anaesthetic placement sites as illustrated at the bottom of the recording sheet. This information was gathered retrospectively from watching videos. Live recording was attempted on the first farm but was difficult to achieve accurately, thereafter, video capture only was used on the farm. Data were entered into an excel spreadsheet for manipulation and statistical analysis.

Statistics for independent variables

Several levels of statistical analysis were performed to investigate the relationships between independent variables and their effects on the outcome variable, “Withdrawal”.

1. Correlation matrix

A correlation matrix was generated to investigate correlations between variables.

2. Logistic regression analysis

Logistic regression analysis was performed using open source logistic regression software to determine which variables were likely to have a significant effect on the binomial outcome (Withdrawal or No withdrawal) after accounting for other variables in the model. Fourteen different iterations of negative stepwise logistic regression were performed. The p-value and odds ratio for each variable from each iteration was determined.

3. Exploration of independent variables

Individual variables were explored in further detail and some relationships were analysed. Basic descriptive statistics were determined. For categorical data, mean and standard deviation of the proportion of antler removals that resulted in a withdrawal response by the stag for different groupings was determined. Comparisons between groups were made using a z-test (2 groups) or chi-squared analysis (more than 2 groups). For continuous data, regression analysis between the proportion of antler removals that resulted in a withdrawal response and the variable value was determined. In addition the difference between different operator “Skill” levels and the variable value was determined and the correlation between operator “Rank” and the variable outcome was determined where appropriate.

Table 2.7 Definitions of variables recorded or calculated to determine which factors affect the likelihood of a withdrawal response during velvet antler cutting in adult stags.

Terms in the column headed “abbreviation” are terms used in the correlation matrix in Figure 2.5 and statistical results in Tables 2.11, 2.12 and 2.13. Variables are grouped according to whether they were a component of the farm, the operator, the animal or the individual antler.

	Variable	Abbreviation	Description and/or unit of variable
Variables related to the farm	Farm	Farm	Identified by a unique letter (A-P)
	Operator	Operator	Identified by a unique number (1-23). The person administering the lignocaine ring block and removing the antler.
	Animal	Animal	Identified by a unique number. The stag.
	Antler	Antler	Identified by a unique number, different from animal number. Most stags had two antlers removed.
Variables related to the operator	Years velveting	YrsVelv	Number of years the operator has been velveting stags.
	Stags velveted/year	NumVelvYear	Approximate average number of stags velveted per year by the operator
	Injection device	Injector Type	2mL vax gun; 5mL vax gun; 20mL syringe; 3 ml vax gun; 35ml syringe; 1ml vax gun.
	Needle length	NeedLength	0.75”, 1.0”, 1.5” length in inches.
	Needle gauge	NeedGauge	16g, 18g, 19g, 20g. Thickness of the needle shank.
	Injects in/end/out	InjectInOut	Deposits local anaesthetic as the needle is being advanced (in), after the needle has been fully inserted (end), or as the needle is being withdrawn (out)
	Depth of needle	Depth	How far the needle is inserted under the skin when the injection is made: <1/3, 1/3to2/3, or >2/3 of needle length.
	Saw type	Saw	Meat saw; Pruning saw; Wood saw; Hydraulic loppers; Other
	“Rank”	Rank	1 to 23. Based on the % of antlers removals that were associated with a withdrawal response. 1=lowest withdrawal response rate, 23= highest withdrawal response rate.
	“Skill”	Skill	“Good”, “Moderate”, “Poor”. Operator groupings based of the % withdrawal response. “Good” <20%, “Moderate” 20 to 40%, “Poor” >50%
Variables related to the animal	Age	Age	Stag age in years
	Sedation	Sedation	Sedated or not (yes or no)
	Primary/regrowth	Regrowth	1: First cut antler or 2: regrowth (second cut)
	Head tied	Tie	Antlers only, nose only, nose+antlers, other, none
	Tourniquet type	Tourniquet	1= new rubber, 2= rubber band, 3=rope, 4=car inner tube, 5=bungy elastic, 6= 4mm rubber tubing 7=stretchy tie-down
	Tourniquet time	TournTime	Time taken to apply tourniquet in seconds (sec)
	Total time	TotalTime	From start of tourniquet application to end of last antler cut in minutes (min)
Antler (Continued next page)	Pedicle circumference	Circumference	Pedicle circumference just below the coronet with a small tape measure (cm)
	Left or right antler	Left/Right	The left or right antler with respect to the animal

Table 2.7 continued

	Variable	Abbreviation	Description and/or unit of variable
Variables related to the antler	Order	Order	1st or 2nd antler to have lignocaine administered
	Proximity	Proximity	Antler on the same or opposite side of the animal to the operator
	Number injections	NumInj	Number of injection sites per antler
	Injection pattern	Pattern	1=U block around the cranial, lateral and caudal sides of the pedicle, 2=arc around less than 3/4 of the pedicle circumference, 3=oval around both antlers together, 4=double oval/circle around each antler individually.
	Volume	Volume	mL of lignocaine injected per antler (mL)
	Dose	Dose	Volume per antler/ pedicle circumference (mL/cm)
	Height of injections	InjHeight	In relation to junction of pedicle and frontal bone High= above, Medium= at to 0.5cm below, Low= >0.5cm below (Fig 2.4b)
	Vol/region 1 to 8	vol 1, vol 2, etc	Figure 2.4a: mL of lignocaine injected at each "region" 1 to 8. Volume estimated from video footage with each "region" assessed individually.
	Total Gaps	Gaps TOT	Estimated number of regions (Fig 2.4a) with at least one gap in the lignocaine ring block. A "gap" is assessed visually from video footage as an subcutaneous region that was not fully covered by lignocaine.
	Gaps / each region 1 to 8	gap1, gap2 etc	Yes (1) or no (0). Subjective assessment of whether a gap occurred in each of "regions" 1 to 8.
	Dose/region 1 to 8	dose 1, dose 2, etc	Vol/region divided by pedicle circumference. This assumes that each "region" is 1/8th of the total circumference. (mL/cm) for each "region".
	Extra nerve block	ZT	Was an additional nerve block given as well as ring block? Injection site is below the zygomatic arch that blocks the zygomaticotemporal (ZT) nerve
	Tissue toughness	Impedance	Difficulty injecting local anaesthetic under skin 0=very easy, 3=can't inject or needle blows off
	Sequential order	sequential	Were the injections placed consecutively or disjointed?
	Wait time	Wait Time	Time from last injection to nick test on that antler
	Cut time	Cut time	Time from start to finish cutting, not including nick test
	Position of nick test	Nicksite	Cranial, craniomedial, medial, caudomedial, etc. see diagram 1
	Direction of cutting	cutdirection	Cran-caud, lat-med, cranlat-caudmed, etc.
	Degree haemorrhage	haemostasis	0=no bleeding, 1=<5drips/ sec, 2=>5drips/sec, 3= constant stream, 4= spurting

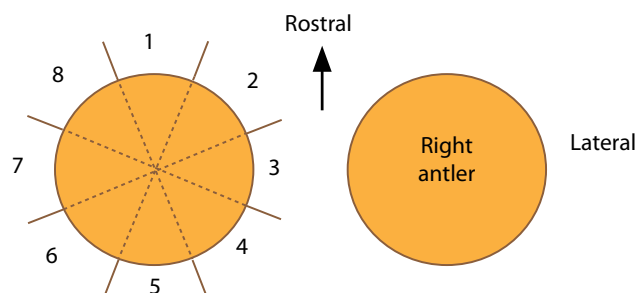


Figure 2.4a Distal-Proximal view of transverse section through antler or pedicle showing regions for recording volume and dose of local anaesthetic, presence of “gaps” and place being cut when a withdrawal response was seen

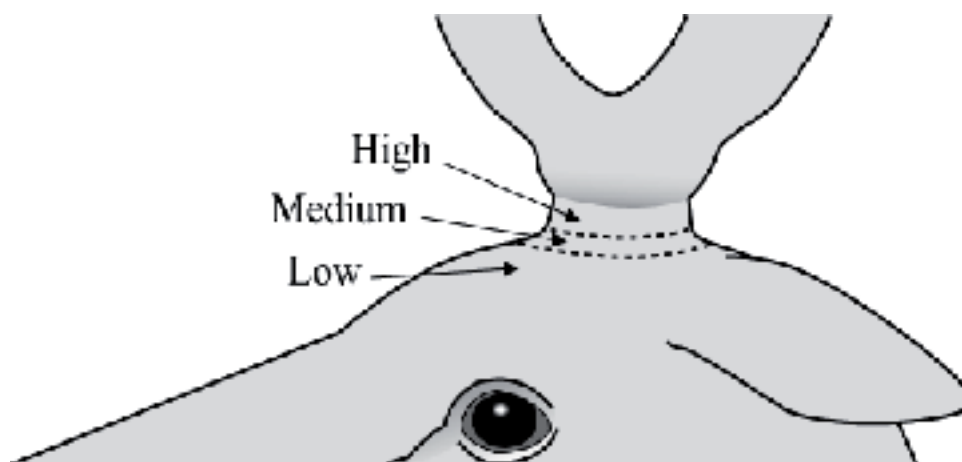


Figure 2.4b Diagram showing high, medium and low level for placement of local anaesthetic ring blocks for velvet removal

4. “Technique model”

A “Technique” model of sequentially added variables relating to specific and statistically significant aspects of injection technique was made based on three different outcome models:

- a) Comparing the effects of independent variables on the withdrawal response. Withdrawal vs no Withdrawal.
- b) By grouping operators into three categories of “Skill” (“good”, “moderate” or “poor”) according to the proportion of withdrawal responses recorded for each operator
- c) By ranking operators from best to worst according to the proportion of withdrawal responses recorded for each and measuring the effect of the correlation analysis.

The three outcome models are shown together graphically (Figure 2.23).

5. “Effects model”

A two-way effects model was generated in an attempt to describe the combination of variables that was best able to separate antlers that had a withdrawal response during cutting from those that did not. This model was extended from the technique model in step 4 to add stag effects in another dimension.

6. Inter-relationship diagram

Finally, a diagram illustrating the inter-relationship of the different factors that may affect the success of analgesia was created.

Results

Overall withdrawal response rate

Out of 455 antlers, a withdrawal response occurred during cutting on 158 (35%) of antlers while 297 (65%) did not meet the criteria for a withdrawal response (Table 2.4).

Behavioural outcomes (dependent variable)

Total number of “movements” recorded

Table 2.8 shows the number of “movements” that included each of the “behaviours”. In total, 3044 “movements” were recorded. Of these, 1159 (38%) occurred during a potentially noxious procedure (pulling on tourniquet, inserting needles, cutting the antler) and 732 occurred during actual antler cutting. For individual animals, the number of “movements” made during the entire period ranged from zero to 63. The number of “movements” made when the animal was not being handled ranged from zero to 50 (mean 10) and the number of “movements” made during cutting of the velvet antler ranged from zero to 46 (mean 3.3). There was no correlation between the number of “movements” made before and during cutting for individual animals.

Occurrence of “behaviours” during potentially noxious stimuli or when a potentially noxious stimulus was not being applied and during antler cutting with and without a withdrawal response.

Table 2.8 also shows the number of “movements” that included each “behaviour” and each “grade” made during a potential noxious stimulus and during times when no stimulus was applied and during antler cutting when a withdrawal response was demonstrated or during velvet antler cutting that was not associated with a withdrawal response. The Z-values and p-values for comparing two proportions are also shown for each “grade” and each “behaviour”.

“Grade” and “Behaviour” during potentially noxious stimuli or when no potentially noxious stimuli were being applied.

“Grade”

“Grades” “slight” and 1 were significantly more likely to occur when a stimulus was being applied whereas “grade” 3 and “look” were more likely to be seen when no stimulus was being applied. There was no significant difference in the proportion of “movements” that occurred during a potentially noxious stimulus or not during a potentially noxious stimulus that were “grade” 2. Of the “movements” that occurred during a potentially noxious stimulus, 36%, 30%, 22%, 11% and 1% were “grade” “slight”, 1, 2, 3 and “look” respectively and of the “movements” that occurred when no noxious stimulus was applied, 23%, 20%, 19%, 18% and 20% were “grade” “slight, 1, 2, 3 and

“look” respectively.

Specific head related “behaviours”

Overall, 32% of “movements” included one or more specific head related “behaviour” (“flinch”, “head arch”, “head down” or “head rotate”). All of these were more likely to occur during a potentially noxious stimulus than at times when no stimulus was being applied. Of the 1159 “movements” that were observed during a potentially noxious stimulus, 40%, 16%, 8% and 9% included “flinch”, “head arch”, “head rotate” and “head down” respectively. Of the 1885 “movements” that were observed when no potentially noxious stimulus was being applied, 2%, 3%, 2%, and 1% included “flinch”, “head arch”, “head rotate” and “head down” respectively.

Occurrence of “grades” and “behaviours” during antler cutting with or without a withdrawal response

The columns on the right of Table 2.8 show “movements” that occurred during antler cutting only. The Z-test and p-values for difference in the proportion of “movements” that included each “behaviour” during antler removal with a withdrawal response or without a withdrawal response is shown.

“Grade”

Of the 732 “movements” that occurred during antler cutting, 42% were grade “slight”, 33% were “grade 1”, 19% were “grade 2”, 6% were “grade 3” and 1% were “look”. There was no difference in the proportion of “movements” that included each “grade” during antler cutting with a withdrawal response versus without a withdrawal response. Of the 675 “movements” that occurred during cutting with a withdrawal response, 41%, 33%, 19%, 5% and 1% were “grades” “slight”, 1, 2, 3 and “look” respectively. Of the 57 “movements” that occurred during antler cutting without a withdrawal response 47%, 28%, 11%, 12%, and 2% were “grades” “slight”, 1, 2, 3 and “look” respectively.

Specific head related “behaviours”

Of the 732 “movements” that occurred during antler cutting, 46% included a “flinch”, 23% included a “head arch”, 10% included a “head rotate” and 12% included a “head down” behaviour. The proportion of “movements” that included specific “behaviours” during antler cutting that included a withdrawal response versus antler cutting without a withdrawal response was higher for “flinch” and “head arch” during a withdrawal response and was not significantly different for “head rotate” and “head down”. Of the 675 “movements” that occurred during antler cutting with a withdrawal response, 46%, 24%, 10% and 12% included “flinch”, “head arch”, “head rotate” and “head down” respectively. Of the 57 “movements” that occurred during antler cutting without a withdrawal response, 16%, 5%, 5% and 9% included “flinch”, “head arch”, “head rotate” and “head down” respectively.

Diagnostic test parameters of each “behaviour” for predicting a potentially noxious stimulus or a withdrawal response during antler cutting

Tables 2.9 and 2.10 show diagnostic test parameters for each “behaviour”. Table 2.9 shows values for predicting whether a potentially noxious stimulus was being applied at the time that each “behaviour” occurred and Table 2.10 shows the values for predicting whether or not a “behaviour” occurred during antler cutting when a withdrawal response occurred or when a withdrawal response did not occur. As the “grade” “behaviours” were mutually exclusive and have usually been combined by previous authors to indicate a withdrawal response, the “grades” “slight”, 1, 2 and 3 were combined for analysis here and were also examined individually along with the “grade” “look”.

Diagnostic test parameters for detecting a potentially noxious stimulus.

The results in Table 2.9 are based on “movements” that occurred when a potentially noxious stimulus was being applied and when no such stimulus was being applied.

For each “behaviour” the following were calculated:

Sensitivity

The number of “movements” that included this behaviour when a stimulus was being applied divided by the total number of “movements” that occurred when a stimulus was being applied. “Flinch”, “head arch”, “head down” and “head rotate” had a sensitivity of 40%, 16%, 9% and 8% respectively for a “movement” that occurred during a potentially noxious stimulus including this “behaviour”. “Grades” “slight”, 1, 2 and 3 combined had a sensitivity of 99%.

Specificity

Specificity was the number of movements that did not include each behaviour when a stimulus was not being applied divided by the total number of movements that occurred when a stimulus was not being applied. “Flinch”, “head arch”, “head rotate” and “head down” had a specificity of 98%, 97%, 99% and 98%, respectively, for a “movement that occurred when no noxious stimulus was applied would not include this “behaviour”. “Grades” “slight”, 1, 2 and 3 combined had a specificity of 20%.

Positive predictive value

The positive predictive value was determined by dividing the number of “movements” that included this “behaviour” that occurred during a potentially noxious stimulus by the total number of “movements” that included this “behaviour”. The positive predictive value for “Flinch”, “head arch”, “head rotate” and “head down” was 94%, 74%, 82% and 71% respectively, for determining whether a movement occurred during a potentially noxious stimulus. “Grades” “slight”, 1, 2 and 3 combined had a PPV of 43%.

Negative predictive value

The negative predictive value was determined by dividing the total number of “movements” that did not include this “behaviour” when no noxious stimulus was being applied divided by the total number of “movements” that did not include this “behaviour. The negative predictive value for

“Flinch”, “head arch”, “head rotate” and “head down” was 73%, 65%, 64% and 68% respectively, for the absence of this “behaviour” indicating that a noxious stimulus was not being applied. “Grades” “slight”, 1, 2 and 3 combined had a NPV of 98%.

Nonspecificity

The nonspecificity was calculated by dividing the number of “movements” that included this “behaviour” that occurred when no stimulus was being applied divided by the total number of “movements” that included this “behaviour”. The nonspecificity for “flinch”, “head arch”, “head rotate” and “head down” was 6%, 26%, 18% and 29% respectively, for a “movement” including this “behaviour” when a stimulus was not being applied. “Grades” “slight”, 1, 2 and 3 combined had a nonspecificity of 50%.

Positive diagnostic likelihood ratio

The PDLR was determined by dividing the sensitivity by 1-specificity. The PDLR for “flinch”, “head arch”, “head rotate” and “head down” was 24.5, 4.65, 7.45, and 4.99 respectively, indicating that these behaviours were this much more likely to occur during a potentially noxious stimulus than when no stimulus was applied. “Grades” “slight”, 1, 2 and 3 combined had a PDLR of 1.23

Negative diagnostic likelihood ratio

The NDLR was determined by dividing the proportion of “movements” that occurred when no noxious stimulus was applied that did not include each “behaviour” by the proportion of “movements” that occurred when a noxious stimulus was being applied and did not include each “behaviour”. The NDLR for “flinch”, “head arch”, “head rotate” and “head down” was 0.61, 0.87, 0.92 and 0.94 respectively. This is how much more likely it is that a “behaviour” will not occur when no stimulus is being applied than when a stimulus is being applied. “Grades” “slight”, 1, 2 and 3 combined had a NDLR of 28.2

Diagnostic test parameters for detecting a withdrawal response during antler cutting.

The results in Table 2.10 are based on “movements” that occurred during antler cutting with and without a withdrawal response.

For each “behaviour” the following were calculated:

Sensitivity

The number of “movements” that included this “behaviour” when a withdrawal response was demonstrated divided by the total number of “movements” that occurred during a withdrawal response. “Flinch”, “head arch”, “head down” and “head rotate” had a sensitivity of 44%, 26%, 12% and 10%, respectively, for a “movement” that occurred during a withdrawal response during antler cutting. For “grades” “slight”, 1, 2 and 3 combined the sensitivity was 99%.

Specificity

Specificity was the number of “movements” that did not include each “behaviour” when the antler was being cut and no withdrawal response was demonstrated divided by the total number of “movements” that occurred during cutting when no withdrawal response was demonstrated.

“Flinch”, “head arch”, “head rotate” and “head down” had a specificity of 84%, 95%, 91% and 95%, respectively, for a “movement” that occurred when no withdrawal response was seen during antler cutting. For “grades” “slight”, 1, 2 and 3 combined, the specificity was 1.8%

Positive predictive value

The positive predictive value was determined by dividing the number of “movements” that included this “behaviour” that occurred during a withdrawal response by the total number of “movements” that included this “behaviour” during antler cutting. The positive predictive value for “flinch”, “head arch”, “head rotate” and “head down” was 97%, 98%, 94% and 96%, respectively, for determining whether a “movement” occurred during a withdrawal response. “Grades” “slight”, 1, 2 and 3 combined had a PPV of 92%.

Negative predictive value

The negative predictive value was determined by dividing the total number of “movements” that did not include this “behaviour” when no withdrawal response was seen during antler cutting divided by the total number of “movements” that did not include this “behaviour during antler cutting. The negative predictive value for “flinch”, “head arch”, “head rotate” and “head down” was 12%, 10%, 8% and 8%, respectively, for the absence of this “behaviour” indicating that a withdrawal response did not occur during antler cutting. “Grades” “slight”, 1, 2 and 3 combined had a NPV of 13%.

Nonspecificity

The nonspecificity was calculated by dividing the number of “movements” that included this “behaviour” that occurred when no withdrawal response was demonstrated divided by the total number of “movements” that included this “behaviour” during antler cutting. The nonspecificity for “flinch”, “head arch”, “head rotate” and “head down” was 3%, 2%, 6% and 4%, respectively, for a “movement” including this “behaviour” when a withdrawal response was not demonstrated during antler cutting.”Grades” “slight”, 1, 2 and 3 combined had a nonspecificity of 8%

Positive diagnostic likelihood ratio

The PDLR was determined by dividing the sensitivity by 1-specificity. The PDLR for “flinch”, “head arch”, “head rotate” and “head down” was 2.93, 4.56, 1.40, and 1.94, respectively, indicating that these behaviours were this much more likely to occur during a withdrawal response during antler cutting than during antler cutting when no withdrawal response was demonstrated. “Grades” “slight”, 1, 2 and 3 combined had a PDLR of 1.01.

Negative diagnostic likelihood ratio

The NDLR was determined by dividing the proportion of “movements” that occurred when no withdrawal response was demonstrated that did not include each “behaviour” by the proportion of “movements” that occurred when a withdrawal response was demonstrated and did not include each “behaviour”. The NDLR for “flinch”, “head arch”, “head rotate” and “head down” was 0.64, 0.80, 0.96 and 0.95, respectively. This is how much more likely it is that a “behaviour” will not occur during cutting when no withdrawal response was demonstrated than during antler cutting when a withdrawal response was seen. “Grades” “slight”, 1, 2 and 3 combined had a NDLR of 1.69.

Table 2.8 Number of movements that included each behaviour type during different phases of velvet antler removal from tourniquet application to antler cutting. Data is broken down into movements that occurred during or between potential noxious stimuli and movements that occurred during antler cutting that were or were not classified as a withdrawal response.

Overall = during entire procedure, **x** = during potentially painful stimulus (tourniquet pull, injecting lignocaine, antler cutting), **o** = not during a potentially painful stimulus; **Cut overall** = during cutting the antler, **Cut/WD** = during cutting and classified as a withdrawal response; **Cut/No WD** = during cutting and not classified as a withdrawal response; **+ve** = the behaviour was part of the movement, **-ve** = the behaviour was not part of the movement. **(%)** = Percent of movements that included this behaviour. Note that all behaviours included one of either slight, grade 1, grade 2, grade 3 or Look and may or may not have included any combination of flinch, HA, HR or HD. * indicates >99% confidence that the difference is not due to chance, Z and p values were calculated from Z-test for two equal proportions.

	Overall	x	o	Cut overall	Cut/WD	Cut/NoWD
Total	3044	1159	1885	732	675	57
Slight +ve	857 28%	420 36%	437 23%	307 42%	280 41%	27 47%
Slight -ve	2187	739	1448	425	395	30
		* Z= 7.738 p<0.01			ns Z= 0.726 p>0.47	
Grade 1 +ve	734 24%	349 30%	385 20%	238 33%	222 33%	16 28%
Grade 1 -ve	2310	810	1500	494	453	41
		* Z= 6.025, p<0.01			ns Z = 0.599, p= 0.549	
Grade 2 +ve	606 20%	252 22%	354 19%	136 19%	130 19%	6 11%
Grade 2 -ve	2438	907	1531	596	545	51
		ns Z=1.939, p=0.052			ns Z= 1.45, p= 0.147	
Grade 3 +ve	471 15%	130 11%	341 18%	43 6%	36 5%	7 12%
Grade 3 -ve	2573	1029	1544	689	639	50
		* Z=5.038, p<0.01			ns Z = 1.784, p = 0.074	
Look +ve	376 12%	8 1%	368 20%	8 1%	7 1%	1 2%
Look -ve	2668	1151	1517	724	668	56
		* Z=15.275, p<0.01			sample proportion too small	
Flinch +ve	498 16%	467 40%	31 2%	321 44%	312 46%	9 16%
Flinch -ve	2546	692	1854	411	363	48
		* Z=27.94, p<0.01			* Z=4.307, p<0.01	
HA +ve	250 8%	185 16%	65 3%	165 23%	162 24%	3 5%
HA -ve	2794	974	1820	567	513	54
		* Z= 12.139, p<0.01			* Z=3.086, p=0.002	
HR +ve	129 4%	92 8%	37 2%	72 10%	69 10%	3 5%
HR -ve	2915	1067	1848	660	606	54
		* Z=7.86, p<0.01			ns Z=0.976, p = 0.329	
HD +ve	134 4%	110 9%	24 1%	88 12%	83 12%	5 9%
HD -ve	2910	1049	1861	644	592	52
		* Z=10.644, p<0.01			ns Z= 0.575, p= 0.565	

Table 2.9 Retrospective diagnostic test parameters of “behaviours” for determining whether a “movement” occurred during a potentially noxious stimulus (tourniquet pull, injecting local anaesthetic or antler cutting) or at times when a stimulus was not being applied

Behaviour	Freq	Sensitivity	Specificity	PPV	NPV	Nonspecificity	PDLR	NDLR
Flinch +ve	0.16	0.40	0.98	0.94	0.73	0.06	24.50	0.61
Flinch -ve								
HA +ve	0.08	0.16		0.74		0.26	4.65	0.87
HA -ve			0.97		0.65			
HD +ve		0.09		0.82		0.18	7.45	0.92
HD -ve	0.04		0.99		0.64			
HR +ve		0.08		0.71		0.29	4.99	0.94
HR -ve	0.04		0.98		0.68			
Look +		0.01		0.02		0.98	0.04	
Look -	0.12		0.84		0.74			1.17
slight +		0.36		0.49		0.51	1.93	
slight -	0.28		0.81		0.86			0.51
grade 1 +		0.30		0.48		0.52	1.82	
grade 1 -	0.24		0.83		0.84			0.61
grade 2 +		0.22		0.42		0.58	1.43	28.2
grade 2 -	0.20		0.85		0.81			
grade 3+		0.11		0.28		0.72	0.77	0.76
grade 3 -	0.15		0.85		0.77			0.96

Stimulus: A potentially noxious stimulus was applied when the movement occurred E.g. pulling the tourniquet, injecting anaesthetic, cutting the antler. This number is the total number of movements that did +(ve) or did not (-ve) include this behaviour in either the stimulus of no stimulus groups

No stimulus: The behaviour occurred as part of a movement during a time between potentially noxious stimuli, when no such stimulus was being applied

Frequency: Proportion of all movements that included this behaviour

Sensitivity: Proportion of movements during a potentially noxious stimulus that include this behaviour

Specificity: Proportion of movements when no stimulus was applied, that did NOT include this behaviour

PPV: Positive predictive value: Proportion of times that this behaviour was seen, that a potentially painful stimulus was being applied

NPV: Negative predictive value: Proportion of times that this behaviour is not seen, that a potentially noxious stimulus was not being applied.

Non specificity: Proportion of movements that included this behaviour that were not during a potentially noxious stimulus

PDCLR: Positive diagnostic likelihood ratio: Proportion of times that this behaviour was seen during a potentially noxious stimulus divided by the proportion of times that it was seen during no stimulus. Ie for every flinch positive in the no-stimulus group, there will by 25 flinch positive animals in the stimulus group.

NDCLR: Ratio of the proportion of times that the behaviour was not be seen during stimulus to the proportion of times that it was not be seen during no-stimulus. E.g. for each animal that does not show a head arch when no stimulus is being applied, 0.87 animals will not show a head arch when a stimulus is being applied

Table 2.10 Diagnostic test parameters of "behaviours" for determining whether a "movement" occurred during antler cutting when a withdrawal response occurred or during antler cutting without a withdrawal response.

Behaviour	Freq	Sensitivity	Specificity	PPV	NPV	Non-specificity	PDLR	NDLR
Flinch +ve	0.44	0.46	0.84	0.97	0.12	0.03	2.93	0.64
Flinch -ve								
HA +ve	0.23	0.24	0.95	0.98	0.10	0.02	4.56	0.80
HA -ve								
HD +ve	0.12	0.12	0.91	0.94	0.08	0.06	1.40	0.96
HD -ve								
HR +ve	0.10	0.10	0.95	0.96	0.08	0.04	1.94	0.95
HR -ve								
look+	0.01	0.01	0.98	0.88	0.07	0.13	0.59	1.01
look -								
slight +	0.42	0.41	0.53	0.91	0.06	0.09	0.88	1.16
slight -								
grade 1 +	0.33	0.33	0.72	0.93	0.08	0.07	1.17	0.96
grade 1 -								
grade 2 +	0.19	0.19	0.89	0.96	0.08	0.04	1.83	0.92
grade 2 -								
grade 3 +	0.06	0.05	0.88	0.84	0.07	0.16	0.43	1.08
grade 3 -								

Withdrawal: The movement was graded as a withdrawal reaction during antler cutting, indicating failure of analgesia

No withdrawal: The movement was seen during antler cutting but was not graded as a withdrawal response because it did not meet the criteria.

Frequency: Proportion of all movements (during antler cutting) that included this behaviour

Sensitivity: Proportion of movement graded as withdrawal, that included this behaviour

Specificity: Proportion of movements graded not-withdrawal that did not include this behaviour

PPV: Positive predictive value: Proportion of times that this behaviour was seen, that were graded a withdrawal response

NPV: Negative predictive value: Proportion of times that this behaviour was not seen, that were graded as No withdrawal response.

Non specificity: Proportion of times that this behaviour is included in a movement, that was not during a withdrawal response

PDLR: Positive diagnostic likelihood ratio: proportion of times the behaviour was seen during a withdrawal response divided by the proportion of times that it was seen during no withdrawal. I.e for every flinch positive in the no-withdrawal group, there will by 2.93 flinch positive movements in the withdrawal group

NDLR: Negative diagnostic likelihood ratio. Ratio of the proportion of times that the behaviour was seen during a not-withdrawal movement to the proportion of times that it was not be seen during a withdrawal movement.

Independent variables

The final list of independent variables that were recorded is shown in Table 2.7 in the materials and methods section and descriptive illustrations for antler or pedicle “Region” and “Injection height” are shown in Figures 2.4a and 2.4b respectively.

Correlation Matrix

Figure 2.5 shows a correlation matrix that was generated to explore correlations and interactions between independent variables and of each independent variable with the outcome variable, “withdrawal”. The numbers within the cells are the spearman r values. The following variables had a correlation coefficients of 0.4 or greater that was statistically significant ($p < 0.05$).

“**Farm**” was positively correlated with “Animal” ($r=0.98$), “Antler” ($r=0.98$), “Operator” ($r=0.95$), “Tourniquet” ($r=0.41$), “StagAge” ($r=0.56$), “Sedation” ($r=0.4$), “Regrowth” ($r=0.69$), and “Skill” ($r=0.43$), and negatively correlated with “NeedGauge” ($r= -0.51$).

“**Animal**” was positively correlated with “Farm” ($r=0.98$), “Antler” ($r=1$), “Skill” ($r=0.41$), “Operator” ($r=0.93$), “Tourniquet” ($r=0.41$), “StagAge” ($r=0.51$), “Regrowth” ($r=0.66$), and negatively correlated with “NeedGauge” ($r= -0.51$)

“**Antler**” was positively correlated with “Farm” ($r=0.98$), “Animal” ($r=1$), “Operator” ($r=0.93$), “Tourniquet” ($r=0.44$), “StagAge” ($r=0.51$), “Regrowth” ($r=0.66$) and negatively correlated with “NeedGauge” ($r= -0.51$).

“**Operator**” was positively correlated with “Farm” ($r=0.95$), “Animal” ($r=0.93$), “Antler” ($r=0.93$), “Tourniquet” ($r=0.41$), “StagAge” ($r=0.51$), “Regrowth” ($r=0.64$), and negatively correlated with “NeedGauge” ($r= -0.51$) and “dose 2” ($r= -0.41$).

“**Rank**” was positively correlated with “Skill” ($r=0.91$) and “gapsTOT” ($r=0.48$) and negatively correlated with “InjectTime” ($r= -0.47$), “NumInj” ($r= -0.48$), “Volume” ($r= -0.45$) and “Dose” ($r= -0.50$). Rank was ordered 1 to 23 = smallest to largest proportion of withdrawal responses for that operator.

“**Skill**” was positively correlated with “Rank” ($r=0.91$), “Farm” ($r=0.43$), “Antler” ($r=0.41$), “Animal” ($r=0.41$), “InjHeight” ($r=0.45$), “gapsTOT” ($r=0.55$) and “gap 8” ($r=0.42$) and negatively correlated with “InjectTime” ($r= -0.54$), “NumInj” ($r= -0.53$), “Volume” ($r= -0.42$), “Dose” ($r= -0.46$), “dose 2” ($r= -0.46$) and “dose 4” ($r= -0.43$).

“**YrsVelv**” was positively correlated with “NumVelvYear” ($r=0.49$), “dose 8” ($r=0.43$), “gap4” ($r=0.43$), and negatively correlated with “dose 3” ($r= -0.49$), “Pattern” ($r= -0.48$), and “TournTime” ($r= -0.43$).

“NumVelvYear” was positively correlated with “YrsVelv” ($r=0.49$), “dose 8” ($r=0.43$) and “gap 4” ($r=0.43$) and negatively correlated with “InjTime” ($r=-0.43$), “Pattern” ($r=-0.48$), and “dose 3” ($r=-0.49$).

“Tourniquet” was correlated with “Antler” ($r=0.44$), “Animal” ($r=0.44$), “Farm” ($r=0.41$), “Operator” ($r=0.41$), “WaitTime” ($r=0.47$) and “gapsTOT” ($r=0.43$).

“Injector type” was correlated with “NeedLength” ($r=0.57$) and “Tie” ($r=0.56$).

“NeedLength” was correlated with “Injector type” ($r=0.57$) and “NeedGauge” ($r=-0.56$).

“NeedGauge” was correlated with “Antler” ($r=-0.51$), “Animal” ($r=-0.51$), “Farm” ($r=-0.51$), “Operator” ($r=-0.53$), “NeedLength” ($r=-0.56$).

“Tie” was correlated with “Injector type” ($r=0.56$) and “Sedation” ($r=0.66$).

“StagAge” was positively correlated with “Antler” ($r=0.51$), “Animal” ($r=0.51$), “Farm” ($r=0.56$), “Operator” ($r=0.51$), “Regrowth” ($r=0.80$), “Circumference” ($r=0.79$), “CutTime” ($r=0.41$), and negatively correlated with “haemostasis” ($r=-0.42$), and “Dose” ($r=-0.52$).

“Sedation” was correlated with “Tie” ($r=0.66$) and “Regrowth” ($r=0.50$).

“Regrowth” was correlated with “Antler” ($r=0.66$), “Animal” ($r=0.66$), “Farm” ($r=0.69$), “Operator” ($r=0.64$), “StagAge” ($r=0.80$), “Sedation” ($r=0.50$), “Impedance” ($r=0.45$), “Circumference” ($r=0.69$), “TotalTime” ($r=0.46$), “haemostasis” ($r=-0.42$) and “Dose” ($r=-0.45$).

“Circumference” was positively correlated with “StagAge” ($r=0.79$), “Regrowth” ($r=0.69$), “Impedance” ($r=0.41$) and negatively correlated with “Dose” ($r=-0.53$).

“Impedance” was correlated with “Regrowth” ($r=0.45$) and “Circumference” ($r=0.41$).

“Left/Right” was not correlated with any other variables.

“Order” was correlated with “Proximity” ($r=0.72$). (First antler was most often nearest side).

“Proximity” was correlated with “Order” ($r=0.72$). (Nearest antler was most often first removed).

“TournTime” was negatively correlated with “NumYrsVelv” ($r=-0.43$).

“InjectTime” was positively correlated with “TotalTime” ($r=0.41$), “NumInj” ($r=0.53$) and negatively correlated with “Rank” ($r=-0.47$), “Skill” ($r=-0.54$) and “NumVelvYear” ($r=-0.47$).

“WaitTime” was correlated with “TotalTime” ($r=0.79$) and with “Tourniquet” ($r=0.47$).

“CutTime” was correlated with “StagAge” ($r=0.41$) and “TotalTime” ($r=0.42$).

	Withdrawal	Rank	Skill	Antler	Animal	Farm	Operator	YrsVelvetng	NumVelvY	Tourniquet	Saw	Injector type	NeedLength	NeedGauge	Tie	StagAge	Sedation	Regrowth	TournTime	Impedence	Left/Right	Proximity	Order	Circumference	InjectTime	Wait Time	CutTime	TotalTime
Withdrawal	1.00																											
Rank	0.39	1.00																										
Skill	0.38	0.91	1.00																									
Antler	0.17	0.33	0.41	1.00																								
animal	0.17	0.33	0.41	1.00	1.00																							
Farm	0.18	0.34	0.43	0.98	0.98	1.00																						
Operator	0.14	0.26	0.37	0.93	0.93	0.95	1.00																					
YrsVelv	-0.06	-0.05	0.14	-0.22	-0.22	-0.19	-0.19	1.00																				
NumVelvY	0.04	0.17	0.27	-0.16	-0.16	-0.10	-0.15	0.49	1.00																			
Tourniquet	0.14	0.23	0.32	0.44	0.44	0.41	0.41	0.31	-0.05	1.00																		
Saw	-0.08	-0.22	-0.09	0.23	0.24	0.25	0.27	0.07	0.18	0.08	1.00																	
Injector	-0.03	-0.02	0.04	0.15	0.16	0.16	0.20	0.20	-0.13	0.39	0.06	1.00																
NeedLeng	-0.02	0.04	0.01	0.16	0.16	0.14	0.18	-0.15	0.11	-0.02	-0.06	0.57	1.00															
NeedGaug	-0.11	-0.22	-0.21	-0.51	-0.51	-0.51	-0.53	0.38	0.03	-0.11	-0.28	-0.32	-0.56	1.00														
Tie	-0.05	-0.16	-0.07	0.16	0.16	0.20	0.20	0.29	-0.11	0.20	0.00	0.56	-0.08	0.02	1.00													
StagAge	0.27	0.35	0.35	0.51	0.51	0.56	0.51	-0.13	0.04	0.23	-0.27	-0.06	-0.13	-0.10	0.11	1.00												
Sedation	-0.10	-0.28	-0.22	0.38	0.38	0.40	0.38	-0.06	-0.39	0.29	-0.08	0.27	-0.12	0.07	0.66	0.24	1.00											
Regrowth	0.18	0.29	0.29	0.66	0.66	0.69	0.64	-0.18	-0.29	0.30	-0.25	0.03	-0.17	-0.02	0.30	0.80	0.50	1.00										
TournTime	-0.03	-0.22	-0.28	-0.05	-0.05	-0.11	-0.02	-0.43	-0.27	0.04	-0.01	0.02	0.13	-0.27	-0.17	-0.01	-0.15	-0.08	1.00									
Impedenc	-0.04	0.05	0.01	0.16	0.16	0.18	0.17	-0.13	-0.23	0.09	-0.14	0.04	-0.11	0.09	0.20	0.29	0.29	0.45	0.05	1.00								
Left1Right	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.00	-0.03	0.06	1.00							
Proximity	-0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	-0.01	0.01	0.22	1.00						
Order	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.72	1.00					
Circumfer	0.17	0.21	0.18	0.33	0.33	0.38	0.36	-0.06	0.10	0.21	-0.21	-0.03	-0.12	-0.04	0.14	0.79	0.27	0.69	0.06	0.41	0.00	0.01	-0.01	1.00				
InjectTime	-0.17	-0.47	-0.54	-0.12	-0.12	-0.17	-0.13	-0.22	-0.47	-0.07	0.00	0.05	-0.13	0.26	0.09	0.04	0.19	0.17	0.31	0.30	0.05	0.06	0.05	0.20	1.00			
WaitTime	0.04	0.09	0.16	0.33	0.33	0.34	0.38	0.17	-0.14	0.47	0.03	0.20	-0.08	-0.12	0.12	0.34	0.16	0.36	0.06	0.12	-0.03	0.04	0.01	0.28	0.12	1.00		
CutTime	0.27	0.16	0.16	0.28	0.28	0.28	0.27	-0.09	-0.12	0.22	-0.07	-0.03	-0.11	-0.06	-0.02	0.41	0.01	0.38	0.15	0.17	0.01	-0.09	-0.09	0.29	0.09	0.22	1.00	
TotalTime	0.13	-0.09	-0.09	0.28	0.28	0.28	0.32	0.01	-0.36	0.38	0.02	0.23	-0.10	-0.04	0.20	0.38	0.25	0.46	0.24	0.21	0.00	-0.01	0.00	0.35	0.41	0.79	0.42	1.00
NumInj	-0.21	-0.48	-0.53	-0.09	-0.09	-0.14	-0.16	-0.28	-0.35	-0.18	-0.06	0.05	0.09	0.06	0.04	-0.03	0.13	0.05	0.22	0.10	0.02	0.02	0.01	0.06	0.53	-0.10	-0.04	0.13
Injheight	0.17	0.30	0.45	0.15	0.15	0.19	0.19	0.05	0.43	-0.01	-0.06	-0.12	-0.06	0.00	0.09	0.20	0.00	0.15	-0.19	0.01	0.05	0.01	0.02	0.14	-0.34	-0.03	-0.01	-0.25
ZT	-0.06	-0.15	0.02	-0.10	-0.10	-0.11	-0.06	0.16	0.14	0.11	0.04	-0.12	-0.07	0.15	-0.10	-0.10	-0.09	-0.13	-0.18	-0.08	-0.01	-0.01	-0.01	-0.14	-0.12	0.06	-0.06	-0.05
sequential	-0.01	-0.13	-0.10	-0.11	-0.11	-0.07	0.00	0.08	0.31	-0.22	0.03	-0.19	-0.06	0.14	0.03	-0.05	0.05	-0.10	-0.12	-0.04	0.05	0.14	0.07	-0.09	-0.16	-0.09	-0.09	-0.16
Pattern	-0.05	-0.21	-0.25	-0.13	-0.13	-0.20	-0.26	-0.48	-0.26	-0.19	-0.07	-0.18	0.12	-0.13	-0.35	-0.16	-0.24	-0.20	0.36	-0.13	0.00	-0.01	0.01	-0.17	0.15	-0.19	-0.06	-0.08
InjectInOu	0.04	0.06	0.03	-0.24	-0.24	-0.23	-0.06	-0.19	0.31	-0.03	0.12	0.00	0.05	-0.13	-0.27	-0.01	-0.37	-0.25	0.29	-0.14	0.00	-0.01	0.00	0.05	-0.02	0.06	0.02	-0.02
depth	-0.04	-0.13	-0.12	0.19	0.19	0.20	0.08	0.02	0.15	0.02	0.09	0.04	-0.01	0.10	0.09	0.16	0.14	0.12	-0.03	0.03	0.00	0.00	0.00	0.06	0.14	0.00	0.07	0.04
Nicksite	0.08	0.09	0.13	0.31	0.31	0.33	0.31	-0.11	0.02	0.21	0.23	0.11	-0.11	-0.11	0.23	0.22	0.24	0.25	-0.06	0.06	0.09	0.09	0.00	0.20	0.01	0.13	0.10	0.19
cutdirectio	0.06	0.07	0.10	0.29	0.29	0.31	0.29	0.06	0.23	0.04	0.27	0.08	0.09	-0.20	0.21	0.10	0.14	0.14	-0.17	-0.02	-0.13	-0.04	0.09	0.12	-0.14	-0.04	-0.01	0.00
haemostasi	-0.11	-0.16	-0.15	-0.28	-0.27	-0.31	-0.31	0.13	-0.01	-0.06	0.04	0.07	0.15	-0.01	-0.12	-0.42	-0.19	-0.42	0.07	-0.15	-0.01	0.01	0.00	-0.34	0.00	-0.13	-0.12	-0.18
Volume	-0.21	-0.45	-0.42	-0.21	-0.21	-0.20	-0.22	0.23	0.28	-0.30	0.21	-0.06	-0.22	0.24	0.36	-0.09	0.17	-0.07	-0.16	0.00	-0.01	-0.02	-0.03	0.05	0.29	-0.18	-0.18	-0.07
Dose	-0.27	-0.50	-0.46	-0.36	-0.36	-0.38	-0.40	0.19	0.17	-0.40	0.30	-0.11	-0.16	0.22	0.15	-0.52	-0.04	-0.45	-0.15	-0.21	-0.01	-0.03	-0.02	-0.53	0.13	-0.33	-0.30	-0.26
dose.1	-0.09	-0.06	-0.06	-0.28	-0.28	-0.26	-0.23	0.35	0.25	-0.02	0.17	-0.05	-0.16	0.28	0.02	-0.38	-0.09	-0.32	-0.19	-0.08	0.05	0.01	0.05	-0.35	-0.09	-0.13	-0.19	-0.21
dose.2	-0.11	-0.38	-0.46	-0.33	-0.32	-0.40	-0.41	-0.26	-0.27	-0.18	-0.07	-0.21	-0.04	0.16	-0.29	-0.27	-0.24	-0.25	0.30	-0.05	0.01	-0.01	0.01	-0.20	0.33	-0.20	0.03	0.01
dose.3	-0.09	-0.23	-0.29	-0.24	-0.24	-0.31	-0.36	-0.49	-0.28	-0.38	-0.11	-0.22	0.02	0.05	-0.28	-0.18	-0.24	-0.21	0.30	-0.09	0.00	0.05	0.06	-0.20	0.27	-0.23	-0.08	-0.10
dose.4	-0.16	-0.39	-0.43	-0.19	-0.19	-0.23	-0.27	-0.40	-0.25	-0.28	0.08	-0.17	-0.06	-0.03	-0.15	-0.20	-0.06	-0.21	0.24	-0.12	-0.02	0.05	0.05	-0.23	0.20	-0.22	-0.09	-0.07
dose.5	-0.13	-0.20	-0.15	-0.18	-0.18	-0.14	-0.14	0.22	0.05	-0.30	0.24	-0.06	-0.16	0.10	0.17	-0.30	0.08	-0.28	-0.27	-0.20	0.03	-0.07	-0.11	-0.36	-0.11	-0.22	-0.23	-0.23
dose.6	-0.17	-0.25	-0.21	-0.03	-0.03	0.00	-0.04	0.15	0.26	-0.29	0.28	0.09	0.03	-0.04	0.33	-0.17	0.15	-0.16	-0.25	-0.13	-0.05	-0.07	-0.06	-0.21	-0.04	-0.22	-0.22	-0.20
dose.7	-0.12	-0.15	-0.01	-0.02	-0.02	-0.02	-0.05	0.35	0.24	0.00	0.31	0.05	-0.14	0.11	0.30	-0.25	0.05	-0.15	-0.31	-0.10	0.00	0.00	0.03	-0.29	-0.03	-0.02	-0.17	-0.08
dose.8	-0.13	-0.28	-0.28	-0.26	-0.26	-0.25	-0.22	0.43	0.38	-0.14	0.05	0.06	-0.05	0.27	0.28	-0.23	0.05	-0.18	-0.14	-0.03	0.02	0.03	0.00	-0.15	0.09	-0.10	-0.17	-0.10
gapsTOT	0.31	0.48	0.55	0.28	0.28	0.33	0.39	0.28	0.27	0.43	0.03	0.16	-0.03	-0.09	0.10	0.31	0.04	0.29	-0.08	0.15	0.00	-0.01	-0.03	0.29	-0.25	0.25	0.18	0.10
gap1	0.24	0.28	0.32	0.16	0.16	0.19	0.18	0.04	0.16	0.10	0.00	0.04	0.04	-0.22	-0.02	0.24	-0.11	0.14	0.03	-0.02	-0.05	-0.09	-0.09	0.15	-0.19	0.08	0.07	-0.01
gap2	0.06	0.25	0.32	0.20	0.20	0.28	0.30	0.28	0.21	0.17	0.06	0.19	-0.02	-0.08	0.26	0.14	0.19	0.15	-0.21	0.05	-0.02	-0.03	-0.04	0.04	-0.29	0.11	0.01	-0.06
gap3	0.05	0.12	0.18	0.12	0.12	0.16	0.24	0.38	0.15	0.32	0.10	0.20	-0.09	0.04	0.28	0.09	0.22	0.13	-0.19	0.05	0.01	-0.03	-0.03	0.09	-0.21	0.16	0.04	0.05
gap4	0.12	0.28	0.34	0.16	0.16	0.19	0.25	0.43	0.27	0.27	0.04	0.14	-0.04	0.07	0.20	0.13	0.09	0.15	-0.23	0.13	-0.01	-0.03	-0.06	0.18	-0.14	0.16	0.09	0.03
gap5	0.20	0.29	0.22	0.18	0.18	0.17	0.19	-0.22	-0.05	0.23	-0.11																	

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“TotalTime” was correlated with “Regrowth” ($r=0.46$), “InjectTime” ($r=0.41$), “WaitTime” ($r=0.79$) and “CutTime” ($r=0.42$).

“NumInj” was positively correlated with “InjectTime” ($r=0.53$), “Pattern” ($r=0.51$), “Volume” ($r=0.51$), “Dose” ($r=0.42$), “dose 2” ($r=0.47$), “dose 3” ($r=0.49$), “dose 4” ($r=0.59$) and negatively correlated with “Rank” ($r= -0.48$), “Skill” ($r= -0.53$), “gapsTOT” ($r= -0.57$), “gap 2” ($r= -0.47$), “gap 4” ($r= -0.47$).

“InjHeight” was correlated with “Skill” ($r=0.45$) and “NumVelvYear” ($r=0.43$).

“Pattern” was correlated with “YrsVelv” ($r= -0.48$), “NunInj” ($r=0.51$), “dose 2” ($r=0.59$), “dose 3” ($r=0.70$), “dose 4” ($r=0.59$), “gapsTOT” ($r= -0.57$), “gap 2” ($r= -0.59$), “gap 3” ($r= -0.64$) and “gap 4” ($r= -0.57$).

“Haemostasis” was correlated with “StagAge” ($r= -0.42$), and “Regrowth” ($r=0.42$).

“Volume” was positively correlated with “Dose” ($r=0.79$), “NumInj” ($r=0.51$), “dose 5” ($r=0.42$), “dose 6” ($r=0.63$), “dose 7” ($r=0.48$), “dose 8” ($r=0.49$) and was negatively correlated with “Rank” ($r= -0.45$) and “Skill” ($r= -0.42$).

“Dose” was positively correlated with “Volume” ($r=0.79$), “NumInj” ($r=0.42$), “dose 4” ($r=0.44$), “dose 5” ($r=0.56$), “dose 7” ($r=0.56$) and “dose 8” ($r=0.48$) and negatively correlated with “Rank” ($r= -0.50$), “Skill” ($r= -0.46$), “StagAge” ($r= -0.52$), “Circumference” ($r= -0.53$), “Regrowth” ($r= -0.45$), “gap 5” ($r= -0.41$) and “gap 6” ($r=0.41$).

“Withdrawal” did not have a correlation coefficient greater than 0.4 with any other variable.

Comparison of individual variables based on outcome (“Withdrawal” vs “No Withdrawal” during antler cutting)

Table 2.11 shows the mean, standard deviation and 95% confidence interval of the mean for independent variables from antlers removed when a stag showed a withdrawal response (“Withdrawal”) and when it did not (“No withdrawal”). Also shown in Table 2.11 is the t-test result and significance level for comparison of the means of “Withdrawal” and “No withdrawal” groups. These analyses were made without correcting for correlations between variables.

The following significant differences were found for the “Withdrawal” group compared to the “No Withdrawal” group:

- “Sedation” was used less often
- “StagAge” was greater
- “Regrowth” occurred more often
- “Circumference” was greater
- “InjectTime” was shorter
- “CutTime” was longer
- “TotalTime” was longer
- “Volume” was less
- “Dose” was less
- “NumInj” was less
- “InjHeight” was higher (lower placement)
- “haemostasis” was lower (less bleeding)
- “vol 4” and “vol 6” were lower
- “dose 2”, “dose 4”, “dose 5”, “dose 6”, “dose 7” and “dose 8” were lower
- “gapsTOT” was higher
- “gap 1”, “gap 4”, “gap 5”, “gap 6”, “gap 7” and “gap 8” occurred more often.

Comparison of individual variables based on operator “Skill”.

Table 2.12 shows the mean and standard deviation of each of the independent variables when they were grouped by operator “Skill”. “Skill” was divided into three categories “Good” (<20% withdrawal responses) “Moderate” (20 to 50% withdrawal responses) and “Poor” (>50% withdrawal responses). The p-values from the t-test result comparing each of the “Skill” groups are also shown.

The following significant differences were found between the different “Skill” groupings:

Compared the “Moderate”, “Good” had less “YearsVelv”, less “NumVelvYear”, lower “StagAge” more “sedated”, longer “InjectTime”, longer “TotalTime”, higher “Volume”, higher “Dose” greater “NumInj”, lower “InjHeight”(higher injection site), fewer “ZT”, higher “vol 1”, higher “vol 2”, higher “vol 3”, higher “vol 4”, higher “vol 7”, higher “vol 8”, higher “dose 1”, higher “dose 2”, higher “dose 3”, higher “dose 4”, higher “dose 7”, higher “dose 8”, fewer “gapsTOT”, fewer “gap 1” fewer “gap 2”, fewer “gap 3”, fewer “gap 4”, fewer “gap 6”.

Compared to “Poor” operators, “Moderate” operators had greater “YearVelv”, greater “NumVelvYear”, more “Sedation”, lower “StagAge”, less “Regrowth”, smaller “Circumference”, longer “InjectTime”, shorter “WaitTime”, shorter “CutTime”, higher “Volume”, higher “Dose”, higher “NumInj”, lower “InnjHeight” (higher injection site), more “ZT”, greater “Depth” (needle inserted further), higher “vol 1”, higher “vol 2”, higher “vol 6”, higher “vol 7”, higher vol 8”, higher “dose 1”, higher “dose 2”, higher “dose 4”, higher “dose 6”, higher “dose 7”, higher dose 8”, fewer “gapsTOT”, fewer “gap 1”, fewer “gap 2”, fewer “gap 5”, fewer “gap 6”, and fewer “gap 8”.

Table 2.11 Mean, Standard Deviation and 95% confidence interval of the mean of variables analysed from adult stags that did and did not show a withdrawal response during antler cutting and t-test result for comparison of the means between stags that did and did not show a withdrawal response.

The columns on the far right shows p-values and significance level for t-tests of comparison of means between withdrawal and no withdrawal antlers. * p<0.5, ** p<0.01, * p<0.001, ns= not significant**

	No withdrawal			Withdrawal			T test for difference	
	Mean	SD	95%CI	Mean	SD	95% CI	p-value	signifi- cance
YrsVelv	8.58	6.46	0.87	7.74	5.93	1.07	0.231	ns
NumVelvYear	203	179	24	217	151	27	0.473	ns
NeedLength	1.21	0.64	0.07	1.18	0.62	0.10	0.722	ns
Sedation	0.16	0.37	0.04	0.09	0.29	0.04	0.020	*
StagAge	3.56	2.62	0.30	5.45	4.24	0.66	0.0000	***
Regrowth	1.18	0.38	0.04	1.34	0.48	0.07	0.0002	***
Left/Right	1.50	0.50	0.06	1.50	0.50	0.08	0.973	ns
Circumference	148	28	3.23	161	41	6.38	0.001	**
Proximity	1.50	0.50	0.06	1.49	0.50	0.08	0.772	ns
Order	1.49	0.50	0.06	1.51	0.50	0.08	0.621	ns
TournTime	28.61	15.02	1.79	27.76	14.42	2.42	0.584	ns
InjectTime	32.15	15.57	1.78	26.61	14.85	2.32	0.000	***
WaitTime	3.24	1.53	0.18	3.37	1.61	0.26	0.422	ns
CutTime	13.59	9.83	1.12	28.00	39.38	6.24	0.0000	***
TotalTime	5.60	2.02	0.24	6.22	2.68	0.44	0.016	*
Volume	15.06	3.82	0.44	13.39	3.80	0.60	0.0000	***
Dose	1.04	0.27	0.03	0.88	0.29	0.05	0.0000	***
NumInj	6.89	2.46	0.28	5.86	1.99	0.31	0.0000	***
Injheight	2.30	0.66	0.08	2.53	0.56	0.09	0.0002	***
ZT	0.06	0.24	0.03	0.03	0.18	0.03	0.142	ns
Impedance	0.23	0.66	0.08	0.18	0.50	0.08	0.326	ns
haemostasis	1.29	1.06	0.12	1.03	1.16	0.18	0.024	*

Table 2.11 cont.

	No withdrawal			Withdrawal			T test for difference	
	Mean	Stdev	95%CI	Mean	StDev	95% CI	p-value	signifi- cance
vol 1	2.34	0.94	0.11	2.25	1.17	0.18	0.418	ns
vol 2	1.09	0.94	0.11	0.96	0.99	0.16	0.159	ns
vol 3	0.66	0.87	0.10	0.54	0.73	0.11	0.134	ns
vol 4	0.99	1.10	0.13	0.69	0.82	0.13	0.001	***
vol 5	2.36	1.22	0.14	2.09	1.55	0.24	0.057	ns
vol 6	2.31	1.28	0.15	1.94	1.36	0.21	0.005	**
vol 7	2.93	1.33	0.15	2.72	1.18	0.19	0.091	ns
vol 8	2.42	1.04	0.12	2.28	1.28	0.20	0.242	ns
dose 1	1.29	0.52	0.06	1.18	0.63	0.10	0.075	ns
dose 2	0.62	0.54	0.06	0.49	0.51	0.08	0.017	*
dose 3	0.38	0.50	0.06	0.29	0.40	0.06	0.036	ns
dose 4	0.56	0.64	0.07	0.37	0.46	0.07	0.0002	***
dose 5	1.32	0.69	0.08	1.12	0.81	0.13	0.010	*
dose 6	1.26	0.65	0.07	1.02	0.69	0.11	0.0004	***
dose 7	1.60	0.70	0.08	1.42	0.68	0.11	0.009	**
dose 8	1.31	0.54	0.06	1.16	0.61	0.10	0.009	**
gapsTOT	2.25	1.51	0.17	3.30	1.64	0.26	0.0000	***
gap1	0.06	0.25	0.03	0.23	0.42	0.07	0.0000	***
gap2	0.54	0.50	0.06	0.60	0.49	0.08	0.176	ns
gap3	0.64	0.48	0.05	0.70	0.46	0.07	0.250	ns
gap4	0.61	0.49	0.06	0.73	0.44	0.07	0.006	**
gap5	0.12	0.33	0.04	0.28	0.45	0.07	0.0001	***
gap6	0.19	0.39	0.04	0.39	0.49	0.08	0.0000	***
gap7	0.03	0.17	0.02	0.13	0.34	0.05	0.0005	***
gap8	0.06	0.23	0.03	0.23	0.42	0.07	0.0000	***

Table 2.12 Mean, standard deviation and p-values from t-test result of variables analysed to determine which factors were different between the techniques of good operators (<20% withdrawal during antler cutting), moderate operators (20 to 50% withdrawal during antler cutting) and poor operators (>50% withdrawal during antler cutting).

The mean (Means) and standard deviation (SDs) values for each variable is shown in each operator “Skill” category. p-values from T-test results for comparison of means between each of the operator “Skill” categories are shown in the columns on the right.

Variable	Means			SDs			T tests for significant differences		
	Good	Mod	Poor	Good	Mod	Poor	Good vs mod	Mod vs poor	Good vs poor
YrsVelv	2.29	12	4.31	3.29	5.30	2.52	0.0000	0.0000	0.0001
NumVelvYear	44	288	174	48	179	45	0.0000	0.0000	0.0000
Sedation	0.27	0.12	0.04	0.45	0.32	0.20	0.0003	0.04	0.0000
StagAge	3.12	3.70	6.81	2.39	2.37	5.02	0.04	0.0000	0.0000
Regrowth	0.14	0.17	0.52	0.34	0.38	0.50	0.41	0.0000	0.0000
Circumference	147	150	166	25	32	42	0.42	0.0001	0.0001
InjectTime	44	29	18.40	16	13	7.17	0.0000	0.0000	0.0000
WaitTime	2.97	3.25	3.71	1.36	1.51	1.79	0.11	0.02	0.002
CutTime	17	16	29	10	14	48	0.48	0.0001	0.01
TotalTime	6.27	5.65	5.70	1.87	2.30	2.59	0.02	0.87	0.08
Volume	16	15	11.20	3.67	3.78	2.15	0.005	0.0000	0.0000
Dose	1.1	1.02	0.72	0.31	0.23	0.24	0.0001	0.0000	0.0000
NumInj	8.5	6.39	4.77	2.64	1.84	1.57	0.0000	0.0000	0.0000
Injheight	2.0	2.33	2.91	0.71	0.58	0.29	0.0000	0.0000	0.0000
ZT	0.00	0.09	0.01	0.00	0.28	0.10	0.002	0.01	0.30
Impedence	0.22	0.20	0.24	0.63	0.60	0.61	0.71	0.54	0.83
depth	2.50	2.49	2.20	0.50	0.93	0.77	0.93	0.01	0.001

Table 2.12 cont.

Variable	Means			SDs			T tests for significant differences		
	Good	Mod	Poor	Good	Mod	Poor	Good vs mod	Mod vs poor	Good vs poor
vol 1	2.12	2.50	2.00	0.70	1.10	0.99	0.001	0.0002	0.35
vol 2	1.72	0.97	0.51	0.91	0.92	0.66	0.0000	0.0000	0.0000
vol 3	1.13	0.44	0.53	1.03	0.67	0.73	0.0000	0.32	0.0000
vol 4	1.75	0.66	0.52	1.11	0.85	0.76	0.0000	0.15	0.0000
vol 5	2.53	2.27	1.97	1.20	1.44	1.16	0.10	0.08	0.001
vol 6	2.26	2.36	1.60	1.03	1.48	0.89	0.55	0.0000	0.0000
vol 7	2.49	3.11	2.55	0.95	1.41	1.03	0.0001	0.0005	0.69
vol 8	2.27	2.72	1.50	0.68	1.20	0.80	0.0005	0.0000	0.0000
dose 1	1.18	1.35	1.07	0.46	0.53	0.68	0.01	0.0001	0.17
dose 2	0.97	0.54	0.23	0.54	0.49	0.32	0.0000	0.0000	0.0000
dose 3	0.64	0.27	0.24	0.56	0.41	0.37	0.0000	0.54	0.0000
dose 4	1.00	0.38	0.25	0.67	0.48	0.39	0.0000	0.02	0.0000
dose 5	1.41	1.24	1.08	0.70	0.73	0.78	0.05	0.08	0.002
dose 6	1.26	1.26	0.83	0.61	0.71	0.49	0.97	0.0000	0.0000
dose 7	1.38	1.68	1.34	0.54	0.72	0.72	0.0002	0.0001	0.64
dose 8	1.26	1.44	0.76	0.40	0.55	0.46	0.002	0.0000	0.0000
gapsTOT	1.27	2.65	3.98	1.16	1.42	1.44	0.0000	0.0000	0.0000
gap1	0.01	0.09	0.33	0.10	0.29	0.47	0.01	0.0000	0.0000
gap2	0.30	0.58	0.78	0.46	0.49	0.42	0.0000	0.0005	0.0000
gap3	0.48	0.71	0.73	0.50	0.45	0.45	0.0000	0.79	0.0003
gap4	0.29	0.75	0.78	0.46	0.43	0.42	0.0000	0.59	0.0000
gap5	0.09	0.16	0.35	0.28	0.36	0.48	0.09	0.0001	0.0000
gap6	0.09	0.23	0.52	0.28	0.42	0.50	0.002	0.0000	0.0000
gap7	0.02	0.08	0.08	0.14	0.27	0.28	0.04	0.84	0.04
gap8	0.00	0.05	0.42	0.00	0.23	0.50	0.02	0.0000	0.0000

Compared to “Poor” operators, “Good” operators had less “YearVelv”, less “NumVelvYear”, more “Sedation”, lower “StagAge”, less “Regrowth”, smaller “Circumference”, longer “InjTime”, shorter “WaitTime”, shorter “CutTime”, higher “Volume”, higher “Dose”, higher “NumInj”, lower “InnjHeight” (higher injection site), greater “Depth” (needle inserted further), higher “vol 2”, higher “vol 3”, higher “vol 4”, higher vol 5”, higher “vol 6”, higher “vol 8”, higher “dose 2”, higher “dose 3”, higher “dose 4”, higher “dose 5”, higher “dose 6”, higher dose 8”, fewer “gapsTOT”, fewer “gap 1”, fewer “gap 2”, fewer “gap 3”, fewer “gap 4”, fewer “gap 5”, fewer “gap 6”, and fewer “gap 8”.

Multiple variable logistic regression

Table 2.13 shows a summary from one of the attempts at logistic regression using negative step-wise removal of variables that did not have a significant effect on the outcome (Withdrawal =1, No withdrawal =0). Several different logistic regression attempts were made and results were inconsistent between these. A full data set from the 14 rounds of logistic regression summarised in Table 2.13 is included in Appendix 2.2. At each step of logistic regression more than one variable was removed. After each iteration of logistic regression, each variable had a coefficient and a p-value indicating the direction and magnitude of the effect of the variable and the significance level. An odds ratio was also calculated for each variable. The mean and 95% confidence interval of the odds ratios and p-values are shown in Table 2.13. In the top portion of the table, the variables that were included in the final iteration of logistic regression are shown. The variables that were removed are shown in the lower portion of the table. The odds ratio and p values shown for variables that were removed are averages over all the iterations of logistic regression that each variable was included in. An estimate of the overall “significance” was made and grouped into high, moderate and low and an estimate of the level of confounding with other variables is also made based on the variation in the logistic regression analysis and results of the correlation analysis above.

The final variables in the logistic regression all had a significant effect on the outcome. These were “gap 7”, “gap 8”, “NumInj”, “gap 1”, “gap 6”, and “Regrowth”.

Descriptive analyses for each variable

Farm (“Farm”)

Figure 2.6 shows a bar graph of the proportion of animals that did and did not show a withdrawal response during antler cutting on each “Farm” ranked in order from least withdrawal responses to most. The proportion of antlers for which the stag showed a withdrawal response during cutting ranged from 7% on Farm O to 73% on Farm P. The mean was 32% and SD was 16%. Farm P was an outlier and all other farms had 50% or fewer withdrawal responses. There were significant differences in outcome between farms. (Chi-squared statistic = 65.92, $p < 0.0001$). The effect of “Farm” was confounded by “Operator” and aspects of technique. Logistic regression showed that farm had a variable effect on withdrawal response. When other variables relating to technique

were included in the logistic regression, the effect of farm was not significant. Overall, the significance of the “Farm” effect was low and the level of confounding was high so “Farm” was not included in further modelling. Most of the variation between farms could be explained by other factors relating to injection technique and the stag.

Operator (“Operator”)

Figure 2.7 shows a bar graph of the proportion of animals that did or did not show a withdrawal response during the removal of each antler by operators ranked from the least withdrawal responses (best) to most withdrawal responses (worst) on the x axis. The proportion of antler removals that resulted in a withdrawal response ranged from 0 to 76% between operators. The mean proportion of withdrawal responses per operator was 32% and SD was 20% and was approximately normally distributed. There was a significant difference between operators in the proportion of antlers that had a withdrawal response (Chi-squared statistic = 77.25, $p < 0.0001$). “Operator” was confounded by other variables relating to technique. When these were accounted for in the logistic regression, operator had a variable effect on the outcome. “Operator” was not included in further modelling. Most of the variation between operators can be explained by factors relating to technique and stag factors.

Number of years velveting (“YrsVelv”)

Figure 2.8a shows a scatter plot of the proportion of antlers for which the stag showed a withdrawal response during cutting versus the number of years each operator had been velveting stags. The linear regression line is also plotted. “YrsVelv” ranged for 0 (first year velveting) to 20 years (mean 8.2). There was no significant correlation between “YrsVelv” and the proportion that showed a withdrawal response during antler cutting ($r^2 = 0.00046$, slope = 0). When “Withdrawal” response was compared with “no withdrawal” response, there was no difference in the mean “YrsVelv”. When operators were grouped by “Skill”, “moderate” operators had been velveting for significantly more years than “good” or “poor” operators. “Good” operators had been velveting for significantly fewer years than “moderate” or “poor” operators. These data were not normally distributed and the sample sizes were small. “YrsVelv” was not included in the further modelling because there is no apparent effect and the data were confounded by other variables.

Number of stags velveted per year (“NumVelvYear”)

Figure 2.8b shows a scatterplot of the number of years that each operator had been velveting stags versus the proportion of antler removals that resulted in a withdrawal response by the stag. “NumVelvYear” from 3 - 615. There was no correlation between “NumVelvYear” and the proportion of stags that showed a withdrawal response during velvet antler cutting. When antlers that did and did not result in a withdrawal response during cutting were compared, there was no significant difference in the mean “NumVelvYear”. When operators were grouped according to “Skill” level (“good”, “moderate” or “poor”), “Good” operators velveted significantly fewer stags per year on

Table 2.13 Summary of logistic regression results for determining which independent variables affect the likelihood of a withdrawal response during velvet antler cutting in adult stags.

The top portion shows the variables that were included in the final logistic regression model and the lower portion of the table shows variables that were removed. For the variables included in the final equation, the odds ratio (OR) 95% confidence interval (CI) of the OR and p-value in the final model are shown. For variables that were removed the mean OR, mean 95% CI of the OR, mean p-value and minimum p-value from the rounds of logistic regression that each variable was included in are shown. In the columns on the right hand side, an overall impression of the level of significance for each variable based on p-values and odds ratios is shown along with the degree of confounding with other variables based on variability when other variables were added or removed and the correlation analysis shown in Figure 2.5.

Variables included in final equation						
Variable	OR	95% CI of OR	p value		Significance	Confounding
gap7	4.166	1.755 - 9.891	0.001		high	low- moderate
gap8	3.042	1.498 - 6.178	0.002		high	low -moderate
NumInj	0.853	0.760 - 0.957	0.007		high	moderate
gap1	2.802	1.450 - 5.416	0.002		high	moderate
gap6	1.816	1.104 - 2.988	0.019		moderate	moderate
Regrowth	2.179	1.310 - 3.623	0.003		moderate	moderate
Variables Removed						
Variable	OR mean	95% CI of OR	p-value mean	p-value min	significance	confounding
gap5	2.043	0.848 - 5.031	0.098	0.018	moderate	moderate
dose.2	1.646	0.694 - 93.857	0.274	0.087	moderate	moderate
InjectTime	0.985	0.962 - 1.008	0.335	0.033	moderate	moderate
gapsTOT	1.040	0.519 - 1.404	0.372	0.109	moderate	moderate
WaitTime	0.773	0.634 - 0.947	0.091	0.000	moderate-high	moderate
dose.5	1.518	0.720 - 43.439	0.354	0.094	low	moderate
dose.8	1.307	0.645 - 19.235	0.437	0.119	low	moderate
InjectInOut	0.684	0.416 - 1.130	0.184	0.067	low	high
Circumference	1.015	0.989 - 1.041	0.280	0.007	moderate	moderate
Volume	0.980	0.630 - 3.524	0.356	0.102	low	moderate
Impedance	0.411	0.227 - 0.754	0.006	0.001	?	moderate
Dose	516.166	0.026 - 946.148	0.533	0.106	low	high
dose.6	1.569	0.383 - 259.869	0.576	0.268	low	moderate
Injheight	1.288	0.728 - 2.297	0.458	0.102	low	moderate
sequential	1.486	0.857 - 2.592	0.190	0.003	moderate	moderate
gap2	0.690	0.221 - 1.873	0.502	0.336	low	low-moderate
Operator	0.907	0.836 - 0.984	0.203	0.004	moderate	high
NeedGauge	0.527	0.330 - 0.846	0.163	0.002	moderate	high
dose.1	7.021	0.299 - 2.188	0.427	0.086	low	moderate
dose.4	2.099	0.212 - 1368.653	0.614	0.436	low	moderate

Table 2.13 cont.

Variable	OR mean	95% CI of OR	p-value mean	p-value min	Significance	Confounding
dose.7	5.952	0.271 - 1936.494	0.512	0.138	low	moderate
StagAge	1.132	0.929 - 1.379	0.343	0.067	moderate	high
ZT	1.718	0.284 - 10.559	0.527	0.390	low	mod
Sedation	0.789	0.237 - 2.656	0.559	0.152	low	high
depth	1.115	0.684 - 1.819	0.707	0.536	low	high
gap4	0.904	0.166 - 3.071	0.987	0.987	low	low-moderate
dose.3	0.851	0.111 - 462.838	0.755	0.574	low	moderate
CutTime	1.033	1.003 - 1.064	0.054	0.018	high	effect of outcome
gap3	0.796					
TotalTime	2.365	1.670 - 3.349	0.000	0.000	high	high
haemostasis	1.153	0.856 - 1.554	0.262	0.098		
Pattern	1.097	0.714 - 1.685	0.722	0.638	low	high
cutdirection	1.015	0.837 - 1.231	0.671	0.440	low	unknown
vol.area7	0.126	0.005 - 3.324	0.304	0.081	low	moderate
vol.area1	0.142	0.005 - 4.062	0.150	0.096	low	moderate
vol.area6	0.267	0.012 - 5.885	0.426	0.247	low	moderate
vol.area4	0.290	0.007 - 12.841	0.519	0.400	low	moderate
vol.area2	0.412	0.014 - 11.780	0.456	0.241	low	moderate
vol.area5	0.595	0.039 - 9.095	0.616	0.279	low	moderate
vol.area3	1.469	0.027 - 80.702	0.635	0.512	low	moderate
vol.area8	1.178	0.062 - 22.477	0.666	0.200	low	moderate
Nicksite	0.953	0.654 - 1.408	0.519	0.467	low	low
Saw	1.026	0.291 - 5.071	0.500	0.250	low	low
TournTime	0.981	0.929 - 1.036	0.473	0.130	low	moderate
Tourniquet	3.139	0.879 - 13.996	0.296	0.049	low	moderate
Tie	1.652	0.284 - 47.390	0.669	0.631	low	moderate
NeedLength	1.152	0.122 - 1320.071	0.913	0.853	low	low
Farm	0.585	0.313 - 12.952	0.662	0.444	low	high
Injector	1.276	0.234 - 49.486	0.884	0.847	low	moderate
animal	0.010	0.000 - 0.231		0.004	low	high
Antler	10.909	2.220 - 53.610		0.003	low	high
Left/Right	0.054	0.009 - 0.307		0.001	low	low
Proximity	1.108	0.346 - 3.550		0.863	low	low
Order	1.107	0.345 - 3.553		0.864	low	moderate
NumVelvYear	1.013	0.980 - 1.048		0.451	low	low
YrsVelv	0.641	0.349 - 1.178		0.152	low	low

average compared to “moderate” and “poor” operators and “moderate” operators velveted significantly more stags per year than “good” or “poor” operators. “NumVelvYear” was not included in the further modelling due to multiple confounding and no apparent effect on outcome.

Total number of stags velveted (“Experience”)

Figure 2.8c shows the “NumVelvYear” multiplied by “YrsVelv”. This gave an estimation of the total number of stags velveted as an indication of the operator’s life “experience”. “Experience” ranged from 3 - 8610. There was no correlation between “Experience” and the proportion of stags that showed a withdrawal response. “Experience” was not included in the further models and comparisons were not made between “Withdrawal” and “no-withdrawal” outcome groups or operator groups based on operator “Skill” because of the skewed nature of the data, small sample sizes and multiple confounding in the data.

Injector Type (“Injector”)

Table 2.14 shows the different injectors used for delivering lignocaine, the number of operators that used each “Injector”, the number of antlers that were blocked using each “Injector” and the proportion of antler removals that resulted in a withdrawal response by the stag. Six different “Injectors” were observed. These included 2ml vaccination gun, 5ml vaccination gun, 20ml disposable syringe, 3ml vaccination gun, 35ml disposable syringe and 1ml vaccination gun. Of these the 2ml vaccination gun was most common. There was no significant effect of “Injector” on the proportion of stags that showed a withdrawal response during antler cutting when calculated across all animals without correcting for confounding variables (Chi-square statistic = 1.004, $p=0.96$). “Injector” was confounded with other factors relating to individual operator technique. “Injector” was included in 2 rounds of logistic regression and did not have a significant effect on withdrawal response. “Injector” was not included in further modelling.

Needle length (“NeedLength”)

Table 2.15 shows the different needle lengths used, the number of operators that used each “NeedLength”, the number of antlers that were anaesthetised using each “NeedLength” and the proportion of these for which the stag showed a withdrawal response during antler cutting. Three different “NeedLengths” were observed: Half inch; one inch; and one and a half inch. The most common was one inch(88%). Less than 10% of animals were injected with a one and a half inch needle which was used by two operators. One operator used a half inch needle to anaesthetise 18 antlers. There was no significant difference between needle lengths in the proportion of stags that showed a withdrawal response. “NeedLength” was not included in further analysis due to unequal group sizes and confounding variables.

Needle gauge (“NeedGauge”)

Table 2.16 shows the number of operators that used each “NeedGauge”, the number of antlers

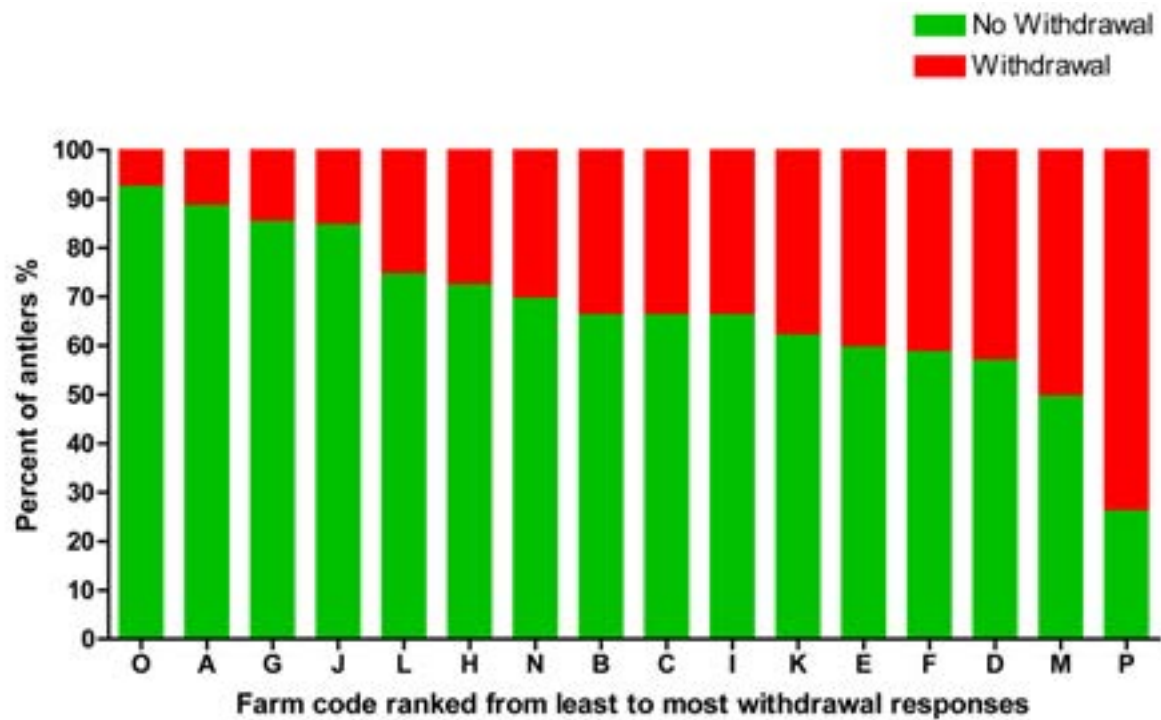


Figure 2.6 Proportion of stags that did or did not show a withdrawal response during cutting each antler on 16 different farms ranked from the lowest percent withdrawal responses (best) to highest percent withdrawal responses (worst)

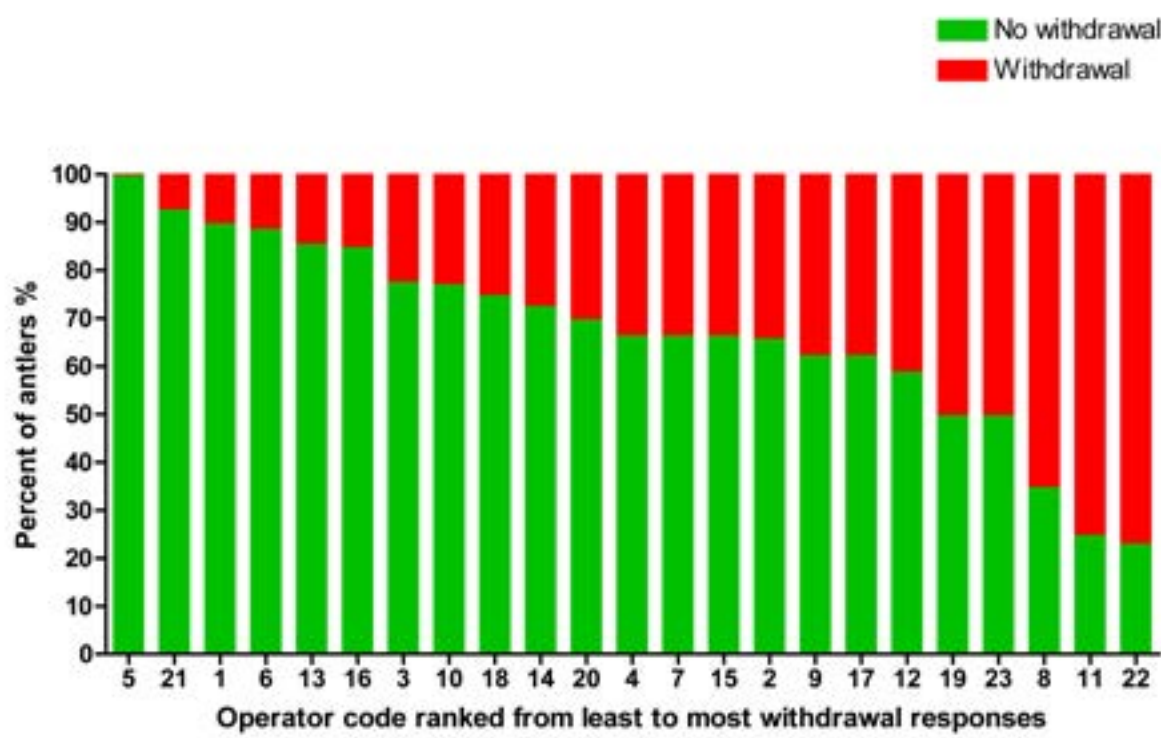


Figure 2.7 Proportion of stags that did or did not show a withdrawal response during cutting each antler for 23 different operators ranked from the lowest percent withdrawal responses (best) to highest percent withdrawal responses (worst)

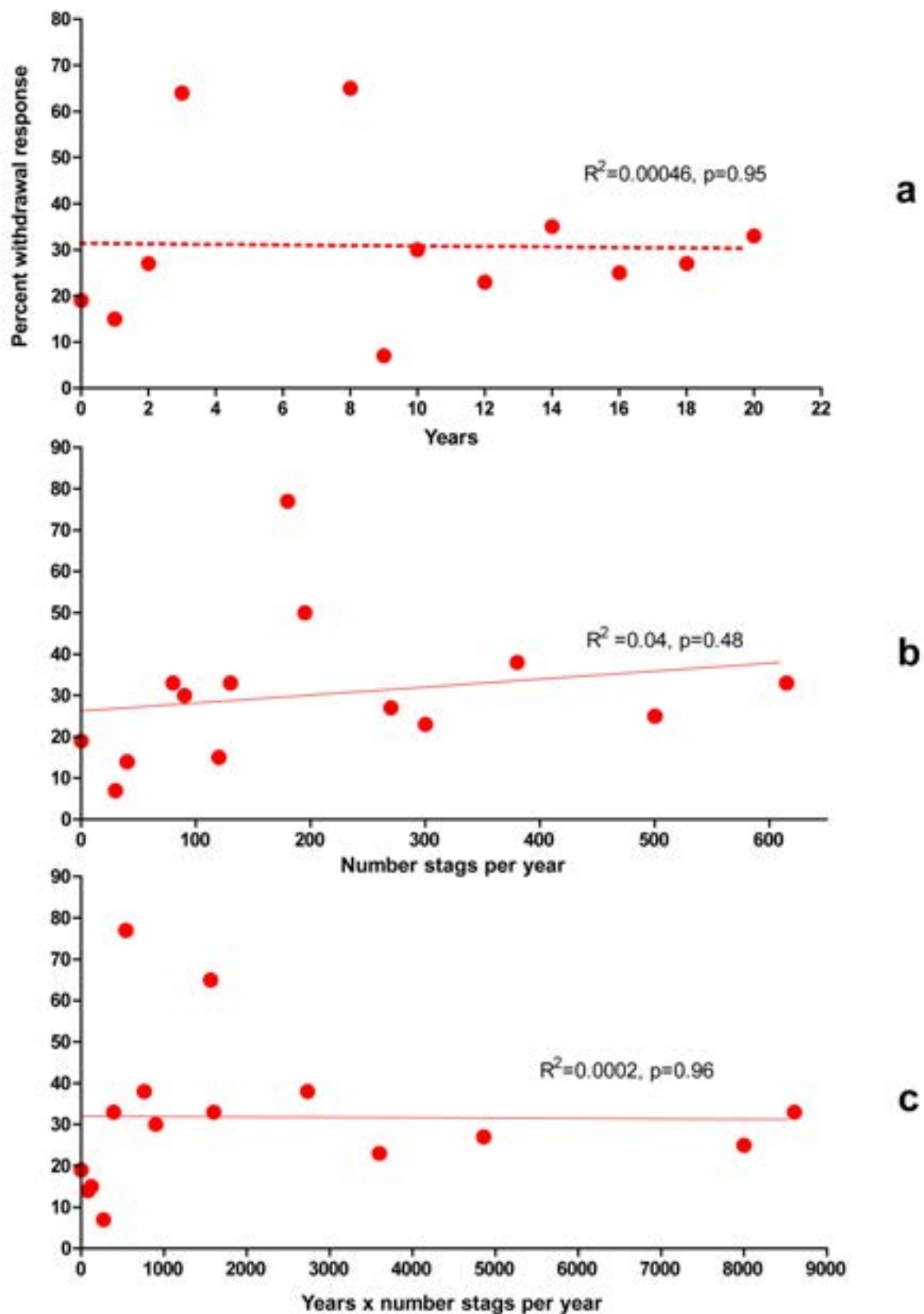


Figure 2.8 Scatterplots of the proportion of stags that showed a withdrawal response during velvet antler cutting versus number of years the operator has been velveting (a), Number of stags velveted per year (b) and total number of stags velveted (Number velveted per year x number of years velveting) (c)

The linear regression line is shown with r^2 value for goodness of fit and p value for probability that the slope is significantly different from zero. $p < 0.05$ indicates 95% chance that the slope is significantly different from zero. None of the slopes shown are significantly different from zero.

Table 2.14 Different types of injection devices used by farmers and veterinarians on commercial farms for injecting lignocaine to provide analgesia for velvet removal from adult stags.

The number of operators that used each device, number of antlers that were blocked and proportion of antlers that showed a withdrawal response during cutting are shown.

Injector type	Number of operators	Number of antlers	Percent withdrawal response
2ml vax gun	14	297	36
5ml vax gun	4	76	36
20ml syringe	1	22	27
3ml vax gun	1	30	30
35ml syringe	1	12	33
1ml vax gun	1	18	33

Table 2.15 Different length needles used by farmers and veterinarians on commercial farms to inject lignocaine to provide analgesia for velvet removal from adult stags.

Number of operators and percent of antlers that each needle length was used by and the percent of animals that showed a withdrawal response during antler cutting.

Needle length	Number of operators	Number of antlers	Percent withdrawal response
1.5"	2	36	35
1"	20	405	31
0.5"	1	18	33

Table 2.16 Different Needle gauges used by farmers and veterinarians on commercial farms for injecting lignocaine to provide analgesia for velvet antler removal from adult stags.

The number of operators that used each needle gauge, percent of antlers blocked and percent of antlers that had a withdrawal response with each needle gauge are shown

Needle gauge	Number of operators	Number of antlers	Proportion withdrawal response
16g	1	18	33%
18g	7	149	44%
19g	1	4	0%
20g	15	288	30%

that were injected with each “NeedGauge” and the percent of antlers for which the deer showed a withdrawal response during cutting. Needle gauge ranged from 20g to 16g with a larger number indicating a finer needle. Because of small numbers in the 16g and 19g sizes, “NeedGauge” was grouped into fine (19g + 20g) and coarse (16g + 18g). There was a significant difference between fine and coarse “NeedGauge” with 42% of antlers cut resulting in a withdrawal response after injection with a coarse needle and 30% withdrawal response after injection with a fine needle (Z statistic = 2.5, $p=0.012$). When “Withdrawal” was compared with “no withdrawal”, on average, “Withdrawal” was associated with coarser needles. When operators were compared based on “Skill” (“good”, “moderate”, “poor”), “poor” operators used significantly coarser needles than “good” operators. When added to the logistic regression “NeedGauge” had a significant effect in some combinations but not others. “NeedGauge” was confounded by “Dose”. Finer gauge needles were used with a significantly higher “dose” of local anaesthetic. Needle gauge was not included in the further analyses.

Stag age (“StagAge”)

Table 2.17 shows the distribution of stag ages and the proportion of antler removals that resulted in a withdrawal response in each age group. “StagAge” ranged from 2 to 14 years with a right skewed distribution. The largest group was 2 year old stags (44%). Twenty eight percent were 3 years old. The remaining 28% of animals were 4 years and older. For analysis of the effects of “StagAge” all stags 4 years and older were grouped. Z tests were performed to test for equal proportions between the three age groupings. There was no significant difference between two and three year old stags in the proportion of antlers for which the stag showed a withdrawal response during cutting. Two and three year old stags both had significantly fewer withdrawal reactions than stags aged 4 years and older ($p<0.01$ and $p=0.04$ respectively).

When “Withdrawal” was compared with “no withdrawal”, the average “StagAge” in the “withdrawal” group was significantly higher than the “no withdrawal” group (5.45 vs 3.56 years respectively) $p<0.00001$.

When operators were compared based on “Skill” level (“good”, “moderate”, “poor”), the average “StagAge” of stags velveted by “poor” operators was significantly older than those velveted by “moderate” and “good” operators (6.81 years, 3.70 years and 3.12 years for “poor”, “moderate” and “good” operators respectively).

When “StagAge” was included in the logistic regression, it sometimes had an effect and sometimes did not have an effect depending on the other variables included. When aspects of injection technique were included in the logistic regression the effect of “StagAge” was reduced.

“StagAge” was confounded by regrowth and “Circumference” and was negatively correlated with “dose”. The operator with the greatest proportion of withdrawal responses was velveting stags over four years of age.

Table 2.17 Age distribution of stags that were observed for behaviour during velvet antler removal. The proportion of stags in each age group that demonstrated a withdrawal response during antler cutting is shown.

Age	Number	Percent of total	Proportion withdrawal response
2	200	44	28%
3	129	28	30%
4	10	2	
5	12	3	
6	16	4	
7	3	0.7	
8	14	3	
9	8	2	48%
10	27	6	
11	4	0.9	
12	14	3	
13	4	0.9	
14	14	3	
Total	455	100	

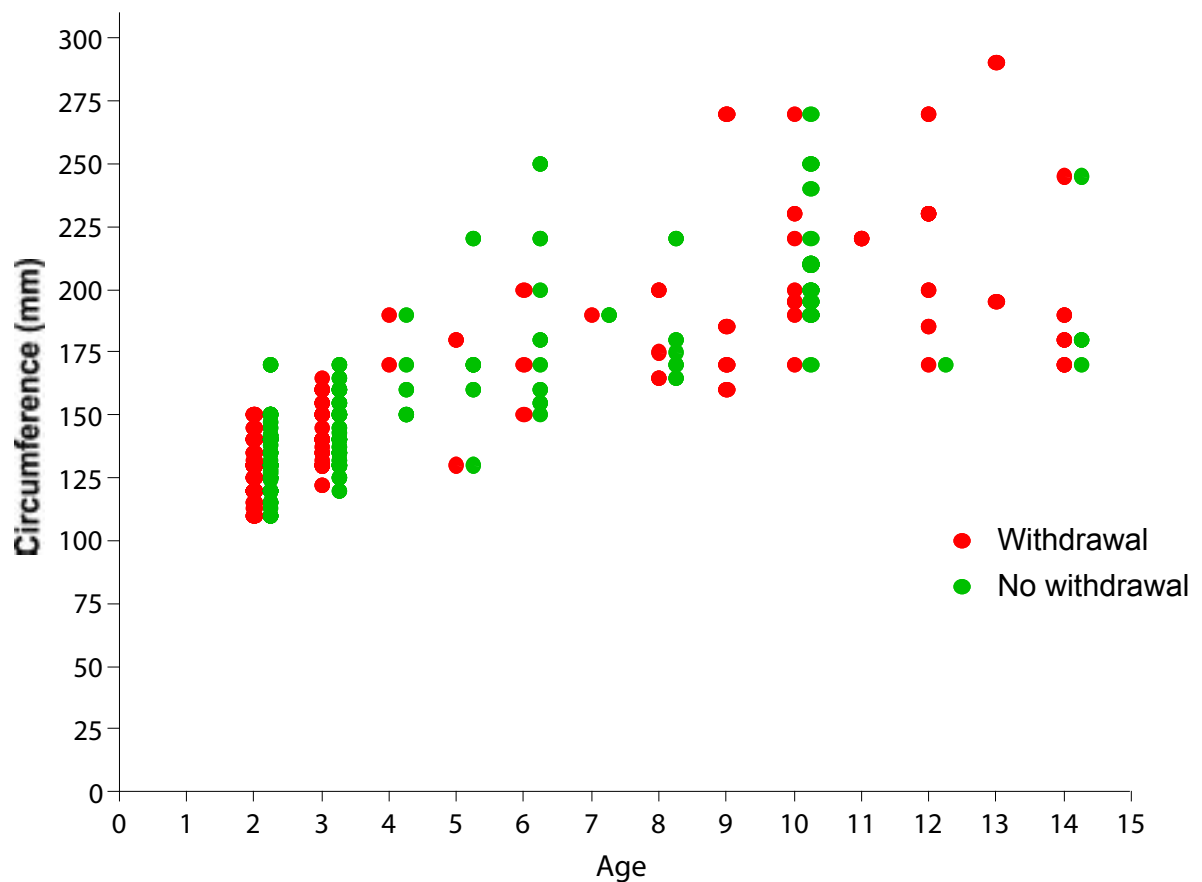


Figure 2.9 Scatterplot of stag age versus pedicle “circumference” showing antlers that the stag showed a withdrawal response during cutting (red) and antlers that the stag did not show a withdrawal response during cutting (green).

Pedicle Circumference (“Circumference”)

Figure 2.9 shows the relationship between “Circumference” and “StagAge”. In Figure 2.9 data have been separated based on withdrawal outcome, (red=“Withdrawal”, green= “no withdrawal”). “Circumference” ranged from 110 to 290mm, the mean was 153mm and SD was 33.8mm.

“Circumference” was positively correlated with “StagAge” (Pearson $r=0.79$), negatively correlated with “dose” (Pearson $r=-0.53$) and stags in regrowth were more likely to have a larger “Circumference”. Antlers in the “Withdrawal” group had a significantly larger mean “Circumference” than antlers in the “no-withdrawal” group (mean “Circumference” 161mm vs 148mm respectively). “Poor” operators were more likely to have antlers with a larger “Circumference” than “good” or “moderate” operators but there was no significant difference between “good” and “moderate” operators. Mean “Circumference” was 147mm, 150 and 166mm for “good”, “moderate” and “poor” operators respectively. Logistic regression showed that “Circumference” did not significantly affect withdrawal outcome when “StagAge” and “Sedation” were included in the logistic regression but did have an effect when these variables were removed. “Circumference” was included the “stag effects” component of the “effects” model because it was easier to separate data points using “Circumference” than “StagAge”.

Sedation (“Sedation”)

Table 2.18 shows the number of antlers removed from stags that were sedated with xylazine or not sedated. Also shown is the proportion of antlers for which the stag demonstrated a withdrawal response during antler cutting for each group. Sixty two antlers were removed from stags that were “Sedated”. A Z-test to compare two sample proportions showed that a significantly smaller proportion of stags showed a withdrawal response during antler cutting in the “Sedation” group than non sedated group (22 vs 36% respectively, $p=0.047$). “Sedation” was confounded by “StagAge”. In the logistic regression analysis, “Sedation” did not have a significant effect on withdrawal when “StagAge” was included. When “StagAge” was removed from the logistic regression analysis, “Sedation” had a significant effect. When “Sedation” was accounted for, “StagAge” had a greater effect on withdrawal response. “Sedation” was included in the “stag effects” component of the “effects” model.

Primary growth/ Regrowth (“Regrowth”)

Table 2.19 shows the number of antlers that were removed as primary antler growth or regrowth and the proportion of these that resulted in a withdrawal response during removal for each group. Also shown in Table 2.19 is the average age for stags that had velvet removed in primary growth and in regrowth. 107 antlers were in the regrowth stage of growth during removal. Stags with regrowth were more likely to show a withdrawal response during antler cutting (30% vs 50% for primary and regrowth respectively, $p=0.0001$). “Regrowth” was related to “StagAge” and “Sedation” and was also confounded by aspects of the operator’s technique. After these factors were accounted for in the logistic regression model, “Regrowth” had a significant effect in some logistic regression equations but not others. “Regrowth” was included in the “stag effects” component of the “effects” model.

Table 2.18 Number of antlers removed from stags that were sedated with low dose xylazine for velvet removal and the proportion of sedated and non-sedated stags that demonstrated a withdrawal response during antler removal.

Sedation	Number	Withdrawal response
No	393	36%
Yes	62	22%

Table 2.19 Number of antlers removed in the primary antler growth or regrowth stage and the proportion of stags that showed a withdrawal response during antler removal. The average age of stags in primary growth and regrowth is also shown.

Stage of antler growth	Number of antlers	Proportion withdrawal response	Average stag age
Primary	348	30%	2.7 years
Regrowth	107	50%	9.1 years

Table 2.20 Number of antlers that were removed from stags with their head restrained or not restrained and the proportion of stags that showed a withdrawal response.

Tied	Number of antlers	Proportion withdrawal response
No	354	36%
Yes	101	29%

Head Tied Down (“Tie”)

Table 2.20 shows the number of antlers that were removed from stags with their head restrained or not restrained and the proportion of these for which the stag showed a withdrawal response during antler cutting. Four different types of head restraints were observed. For the purposes of analysis these were grouped so that stags were either tied or not tied. One hundred and one antlers were removed from stags that had their head tied in some way. The difference in the proportion of withdrawal responses between animals that did or did not have their head tied was not statistically significant when a z-test to compare two proportions was applied. “Tie” did not have a significant effect in the logistic regression (29% vs 36% withdrawal response for “Tied” vs not “Tied” respectively, $p=0.19$).

Tourniquet type (“Tourniquet”)

The following tourniquet types were observed in use:

1. New Rubber tourniquet (advocated by DINZ, available from retail outlets)
2. Rubber band
3. Rope
4. Car Inner tube
5. Bungy elastic cut to length
6. 4mm rubber tubing
7. Motorbike stretchy tie down.

“Tourniquet” did not have a significant effect on the proportion of stags that demonstrated a withdrawal response.

Tourniquet application time (“TournTime”)

“TournTime” was removed from analysis as it was not considered to be a plausible contributing factor to withdrawal response during antler cutting and its inclusion increased confounding in the data.

Injection time (“InjectionTime”)

Figure 2.10 shows “InjectionTimes” for antlers in the “Withdrawal” and “no Withdrawal” groups in the top panel, “InjectionTimes” used by “good”, “moderate” and “poor” operators in the middle panel and “InjectionTimes” for individual operators ranked from best (1, least withdrawal reactions) to worst (23, most withdrawal reactions) in the lower panel. Antlers in the “Withdrawal” group had a significantly shorter “InjectionTime” than antlers in the “no Withdrawal” group. There was a significant difference in “InjectionTime” between “good”, “moderate” and “poor” operators. “Good” operators had significantly longer “InjectionTime” than “moderate” or “poor” operators and “moderate” operators had significantly longer “InjectionTime” than “poor” operators. There was a significant correlation between operator “Rank” and “InjectionTime” with better operators tending to use a longer “Injection” than poorer operators. “InjectionTime” was correlated with

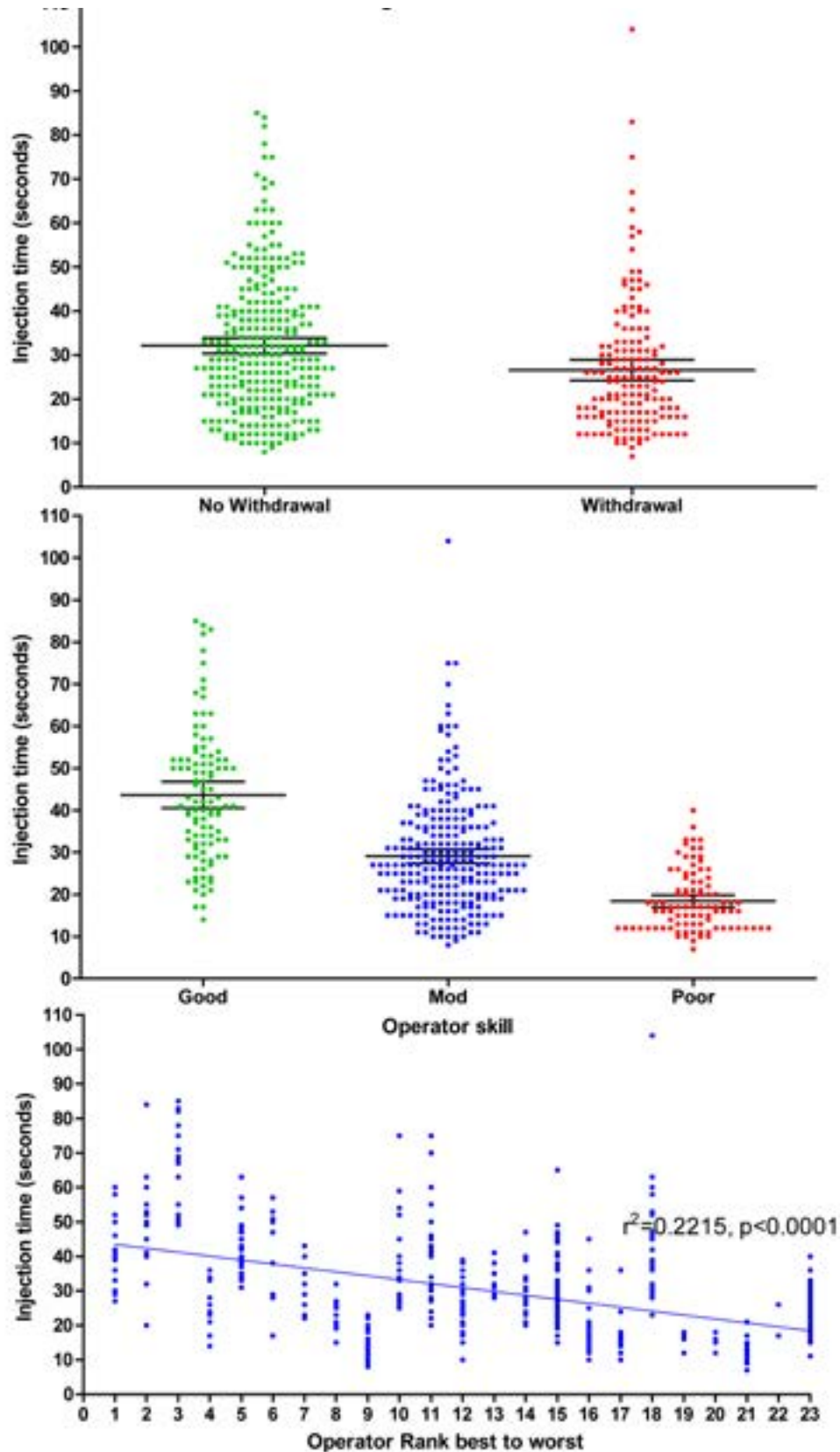


Figure 2.10 Scatterplots of the time taken to inject local anaesthetic around each antler for antlers for which the stag did or did not demonstrate a withdrawal response during removal (top), operators who were grouped as good, moderate or poor "Skill" (middle) and by operator "Rank" from best to worst (bottom). Lines indicate mean and standard deviation in the upper two graphs and the lower graph also shows the pearson r^2 coefficient and p value for likelihood that the slope is equal to zero.

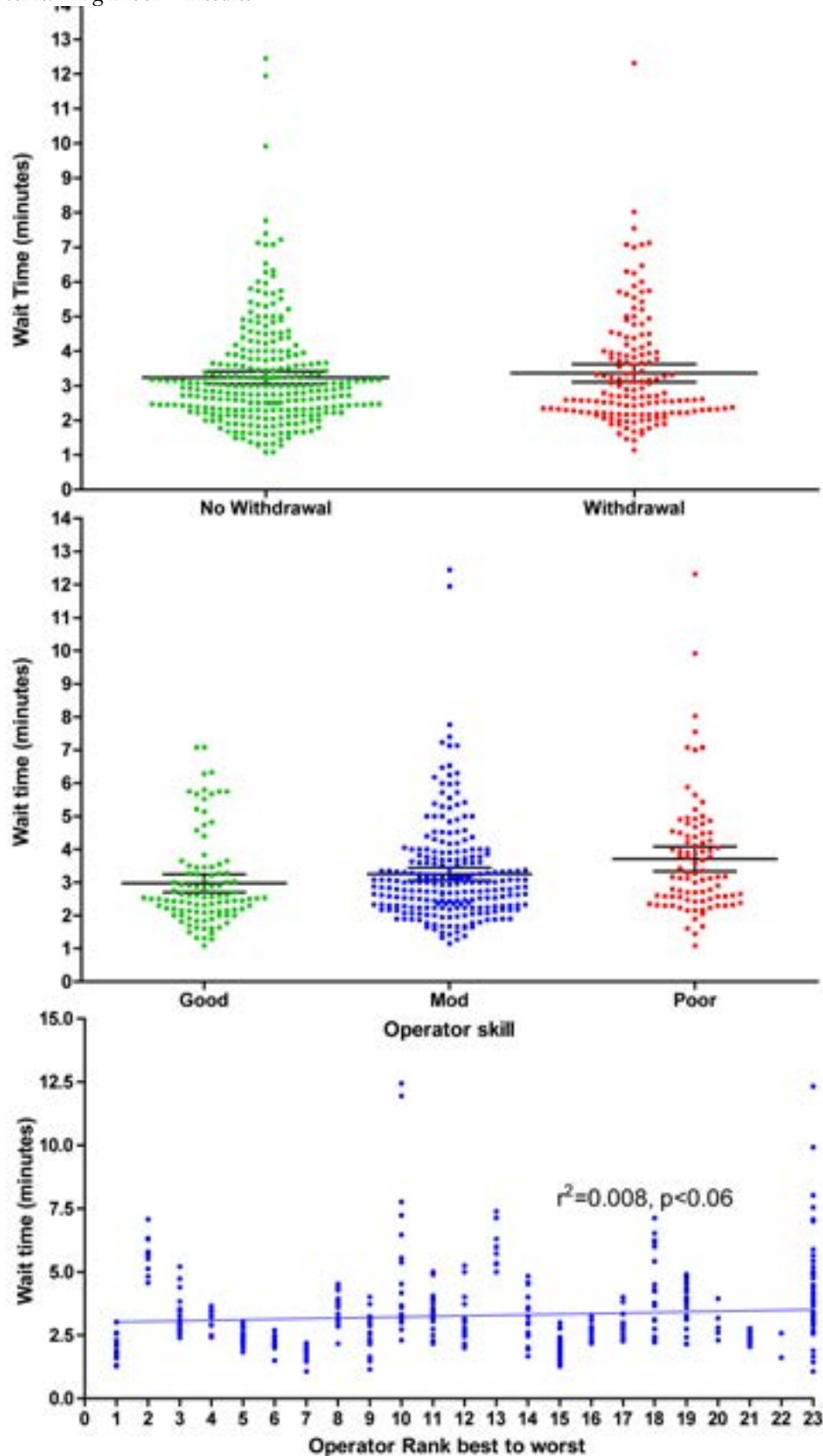


Figure 2.11 Scatterplots of the time waited after injection of local anaesthetic and before antler removal for antlers for which the stag did or did not demonstrate a withdrawal response during removal (top), operators who were grouped as good, moderate or poor "Skill" (middle) and by operator "Rank" from best to worst (bottom). Lines indicate mean and standard deviation in the upper two graphs and the lower graph also shows the pearson r^2 coefficient and p value for likelihood that the slope is equal to zero.

“NumInj” (Pearson $r = 0.53$). When “NumInj” was included in the logistic regression model, “InjectionTime” became less significant.

“Injection time” was included in the “Technique” model and the “Effects” model as a component of technique.

Wait time (“WaitTime”)

Figure 2.11 shows “WaitTime” for antlers in the “Withdrawal” and “no Withdrawal” groups in the top panel, “WaitTimes” used by “good”, “moderate” and “poor” operators in the middle panel and “WaitTimes” for individual operators ranked from best (1, least withdrawal reactions) to worst (23, most withdrawal reactions) in the lower panel.

“WaitTime” did not have a significant effect on withdrawal response. There was no difference in “WaitTime” between antlers in the “Withdrawal” and “No Withdrawal” groups. There was no difference in “WaitTime” between operator “Skill” groups and there was no significant correlation between “WaitTime” and operator “Skill”. “WaitTime” was not included in the further analysis.

Cut time (“CutTime”)

Mean “CutTime” was significantly longer for antlers for which the stag demonstrated a withdrawal during cutting compared to antlers that were not associated with a withdrawal response. There was a significant difference in “CutTime” between “moderate” and “poor” operators but not between “good” and “moderate” operators. There was no significant correlation between “CutTime” and operator “Rank”. “CutTime” had a variable effect on the logistic regression analysis and was removed after 4 rounds. “CutTime” was considered to be influenced by the stag showing a withdrawal response and was not included in further analyses.

Total time (“TotalTime”)

Antlers that were graded as withdrawal had significantly longer “total time” from tourniquet application to removal of the second antler. “Good” operators took significantly longer “total time” than “moderate” operators but not “poor” operators. “Total time” did have a significant effect on the logistic regression model in the early stages of the modelling but was removed because of the mixed results in other analyses and probable correlation with “wait time” and “cut time”.

Antler side and order

Figure 2.12 shows stacked column graphs of the proportion of antlers that the stag did (red) or did not (green) show a withdrawal response during cutting for left vs right antler, first vs second antler and the antler on the same side vs opposite side of the crush as the operator.

Left or Right antler (“Left/Right”)

There was no difference in the withdrawal response between left and right antlers. “Left/Right” was not significantly correlated with any other variable and had no significant effect on logistic regression analysis. More antlers were removed with the operator standing on the left side (61%) compared to the right (39%) and this difference was statistically significant ($p < 0.05$).

First vs second antler (“Order”)

There was no difference in the withdrawal response between first and second antlers. “Order” was not significantly correlated with any other variable except “Proximity” and had no significant effect on logistic regression analysis. First antler was more likely to be on the same side of the crush as the operator.

Proximity to Operator (“Proximity”)

There was no difference in withdrawal response between antlers that were on the same side of the crush as the operator (near) versus those on the other side of the crush (far). “Proximity” was cor-

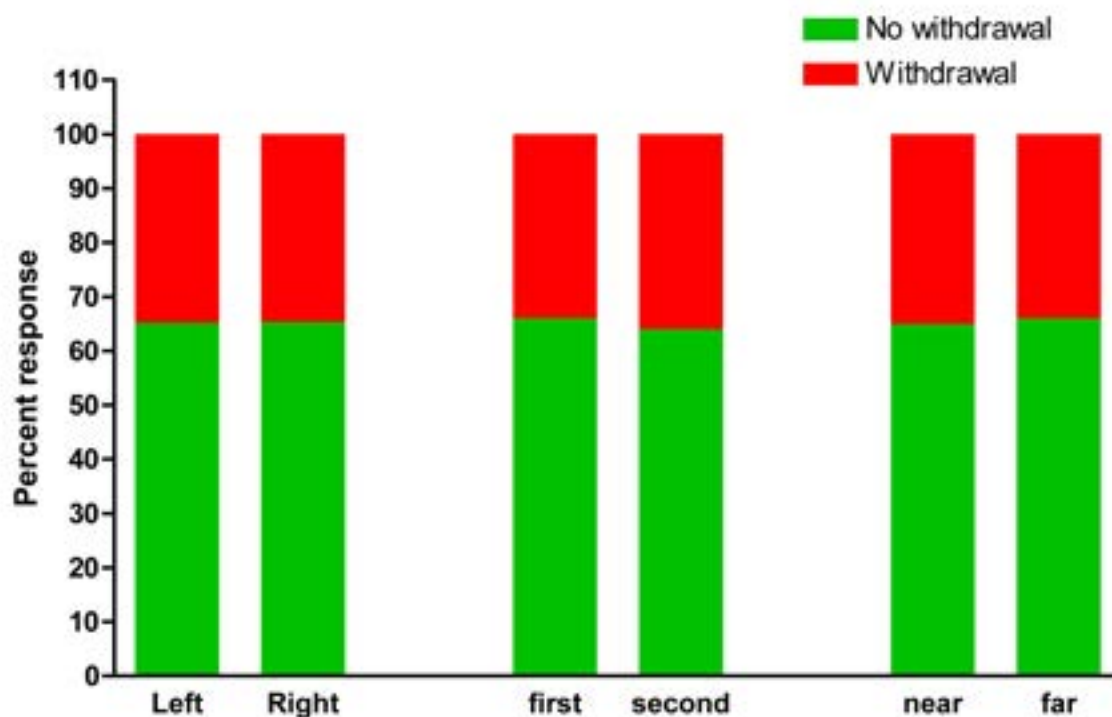


Figure 2.12 Percent of antlers for which the stag did (red) or did not (green) show a withdrawal response during antler cutting. Antlers were grouped by either left or right antler, first or second antler to be cut or antler closest to the operator (near) or furthest away from the operator (far)

related with “Order” with the first antler removed more likely to be the one closest to the operator. 86% of the first antlers to be removed were on the same side of the crush as the operator.

Number of Injections (“NumInj”)

Figure 2.13 shows the “NumInj” for antlers for which the stag did and did not show a withdrawal response during cutting (top panel), the mean and range of “NumInj” for “good”, “moderate” and “poor” operators (middle panel) and the average and standard error of “NumInj” given by individual operators ranked from best to worst (lower panel). “NumInj” ranged from three to 26 and the distribution was skewed to the right. The average “NumInj” per antler was 6.5. Significantly greater “NumInj” were used in antlers for which the stag did not show a withdrawal response compared to antlers that did show a withdrawal response. (mean 6.9 vs 5.9 respectively, $p < 0.0001$). “Good” operators had significantly greater “NumInj” than “moderate” or “poor” operators and “moderate” operators used significantly greater “NumInj” than “poor” operators (mean 8.5, 6.4 and 4.8 for “good”, “moderate” and “poor” operators respectively, $p < 0.0001$). “NumInj” was significantly correlated with operator “Rank”. “NumInj” had a significant effect on withdrawal response in some logistic regression analyses but when the positively correlated variables, “InjTime” and “Dose” were accounted for, these other factors were more significant than “NumInj”. “Number of injections” was included in the “Technique” and “Effects” models.

Volume (“Volume”)

Figure 2.14 shows graphs of the “Volume” of local anaesthetic used for individual antlers. In the top panel antlers for which the stag did or did not have a withdrawal response are shown, in the middle panel antlers removed by “good”, “moderate” or “poor” operators and shown and in the lower panel the “Volume” used for individual operators ranked from best to worst are shown. The total “Volume” ranged from 6.5 to 32.5 mL (Mean 15mL). A significantly higher mean “Volume” was used in the “No Withdrawal” group than the “Withdrawal” group (15.06mL vs 13.39mL respectively). “Good” operators used significantly higher “Volume” than “moderate” or “poor” operators and “poor” operators used significantly lower “Volume” than “moderate” operators. There was a significant correlation between operator “Rank” and “Volume” with better operators using a higher “Volume”. In logistic regression, “Volume” had a significant effect in some combinations of variables but not others. “Volume” and “Dose” were positively correlated (Pearson $r = 0.79$). The variation seen with “Volume” can be mostly explained by “Dose”. “Dose” was included in the “Technique” model and “Volume” was not included.

Volume per Region (“vol 1”, “vol 2” “vol 8”)

Table 2.21 shows the mean, maximum and minimum “Volume per region” for each “Region” (“Regions 1 to 8 are shown in Figure 2.4a). Figure 2.15 shows graphically the mean volume injected at each “Region” for antlers that did and did not demonstrate a withdrawal response in the top panel. The lower panel shows the mean volume injected in each “Region” by “good”, “moderate” and

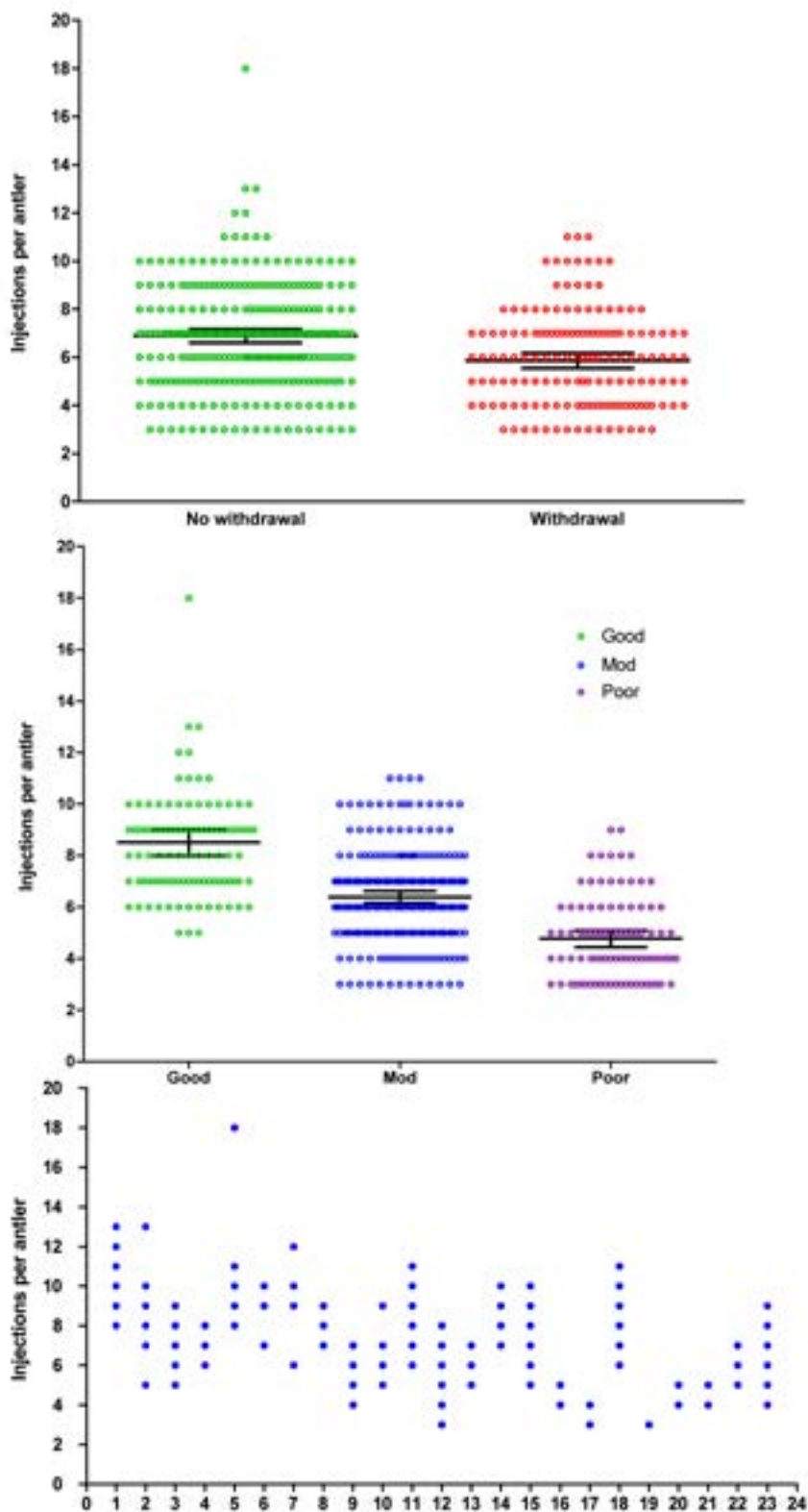


Figure 2.13 Number of injections of lignocaine administered around the pedicle of each antler by farmers and vets to provide analgesia for velvet removal from mature stags on commercial farms.

Top: Number of injections that were given on antlers that did or did not result in a withdrawal reactions during antler cutting. Centre: Number of injections given based on operator “Skill”. “good” operators had 0 to 22% withdrawal response, Moderate operators had 23 to 41% withdrawal responses and poor operators had 50% or more. Bottom: Number of injections per antler by operator rank based on percent withdrawal response 1=least withdrawal responses, 23=most withdrawal responses. Lines indicate mean and standard deviation in the upper two graphs.

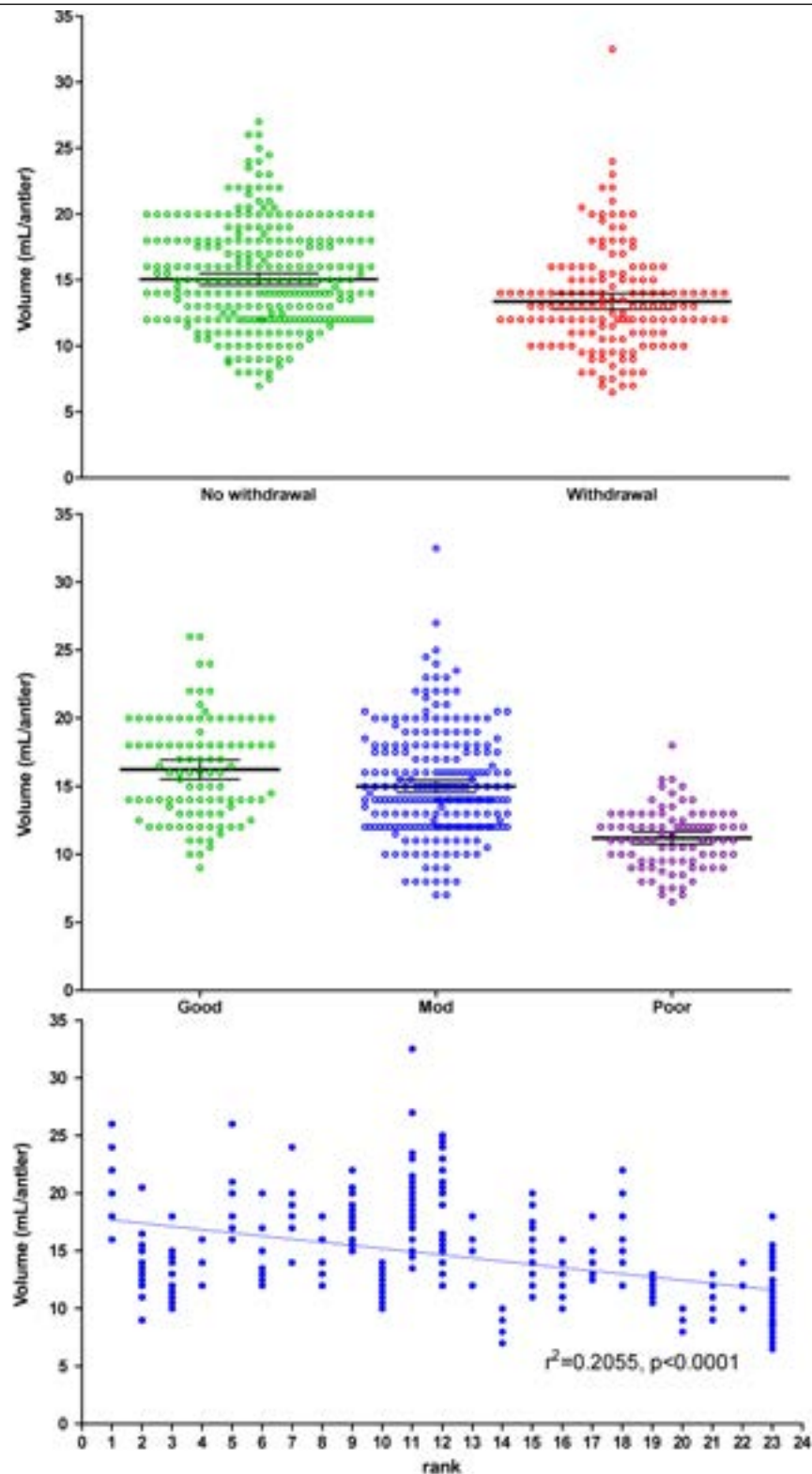


Figure 2.14 Volume of lignocaine injected around each antler pedicle by farmers and veterinarians to provide analgesia for velvet removal from mature stags on commercial farms.

Top: Volume that was injected around antlers that did or did not result in a withdrawal reactions during antler cutting. Centre: Volume injected based on operator “Skill”. Good operators had 0 to 22% withdrawal response, Moderate operators had 23 to 41% withdrawal responses and poor operators had 50% or more. Bottom: Volume injected by operator rank based on percent withdrawal response 1=least withdrawal responses, 23=most withdrawal responses. Lines indicate mean and standard deviation in the upper two graphs and the lower graph also shows the pearson r^2 coefficient and p value for likelihood that the slope is equal to zero.

“poor” operators. Significant differences are indicated by stars. There was no difference between antlers in the “Withdrawal” vs “No Withdrawal” groups in the mean volume injected at “Regions” 1, 2, 3, 5, 7 and 8. Significantly higher mean volume was injected in “Regions” 4 and 6 in the “No Withdrawal” group than the “Withdrawal” group. “Good” operators injected a significantly higher average volume than “moderate” operators in “Regions” 2, 3 and 4 and a significantly lower volume than “moderate” operators in “Regions” 7 and 8. “Moderate” operators injected a significantly higher volume than “poor” operators in “Regions” 1, 2, 6, 7 and 8, but not in “Regions” 3, 4 or 5.

Dose (“Dose”)

Figure 2.16 shows the “Dose” used for individual antlers. In the top panel the “Dose” used on antlers for which the stag did and did not show a withdrawal response is shown, in the middle panel the “Dose” used by “good”, “moderate” and “poor” operators is shown and in the lower panel the “Dose” used on each antler for individual operators ranked from best (least withdrawal reactions) to worst (most withdrawal reactions) is shown. Overall, “Dose” ranged from 0.3mL/cm to 1.96mL/cm (Mean 0.98mL/cm).

Table 2.21 Mean, maximum and minimum volume of local anaesthetic injected by farmers and veterinarians in each of the eight “Regions” around the antler pedicle (as shown in Figure 2.4a) for velvet removal from adult stags on commercial farms.

Region	Mean volume (mL)	Min (mL)	Max (mL)
1	2.31	0	7
2	1.05	0	4
3	0.62	0	4
4	0.88	0	5
5	2.27	0	10
6	2.18	0	9
7	2.85	0.5	8
8	2.37	0	7

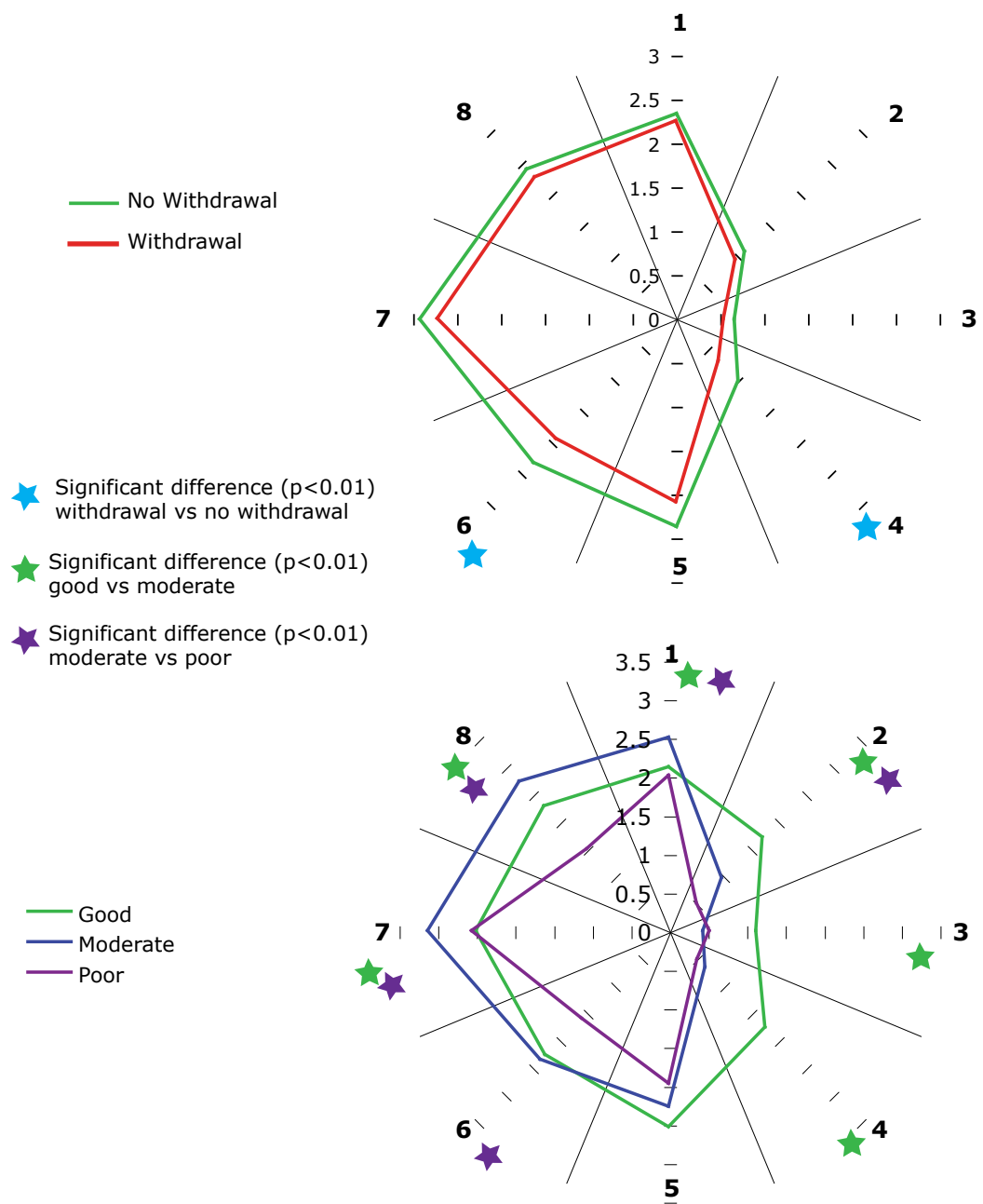


Figure 2.15 Schematic diagrams showing the volume of lignocaine injected in each antler “Region” (as shown in Figure 2.4a) by farmers and veterinarians to provide analgesia for velvet antler removal from mature stags on commercial farms.

The top graph shows the average volume used in each “Region” for antlers that showed a withdrawal response (red) and did not show a withdrawal response (green). The lower graph shows the average volume use in each regions by good (green), moderate (blue) and poor (purple) operators. Significant differences ($p < 0.01$) are indicated by stars.

The mean “Dose” was significantly higher in antlers in the “No Withdrawal” group than the “Withdrawal” group (mean “Dose” 1.04mL/cm vs 0.88mL/cm respectively, $p < 0.0001$). “Good” operators used a significantly higher “Dose” than “moderate” or “poor” operators and “moderate” operators used a significantly higher “dose” than “poor” operators. The mean “Dose” used was 1.10, 1.02 and 0.72mL/cm for “good”, “moderate” and “poor” operators respectively. “Dose” was positively correlated with “Volume” and negatively correlated with “StagAge” and “Circumference”. “Dose” was better at explaining the outcome than “volume” and was included in the “Technique” model.

Dose per pedicle “Region” (“dose 1”, “dose 2” “dose 8”)

Figure 2.17 shows the estimated dose (mL/cm) of lignocaine administered at each of the pedicle “Regions” 1 to 8. (For a diagram of “Regions” see Fig 2.4a). In the top panel of Figure 2.17 is shown the average dose rates for each “Region” in antlers for which the stag did and did not show a withdrawal response during antler cutting. The lower panel shows the average dose given at each antler “Region” by “good”, “moderate” and “poor” operators. Significant differences are indicated with stars. Antlers in the “Withdrawal” group had a significantly lower average “dose 4”, “dose 5”, “dose 6”, “dose 7” and “dose 8” than antlers in the “No Withdrawal” group. “Good” operators had significantly higher “dose 2”, “dose 3” and “dose 4” and significantly lower “dose 7” and “dose 8” than “moderate” operators. “Good” operators had significantly higher “dose 2”, “dose 3”, “dose 4”, “dose 6” and “dose 8” than “poor” operators. “Moderate” operators had significantly higher “dose 1”, “dose 2”, “dose 6”, “dose 7” and “dose 8” than “poor” operators. There was no difference in “dose” used between operators in “Region” 5.

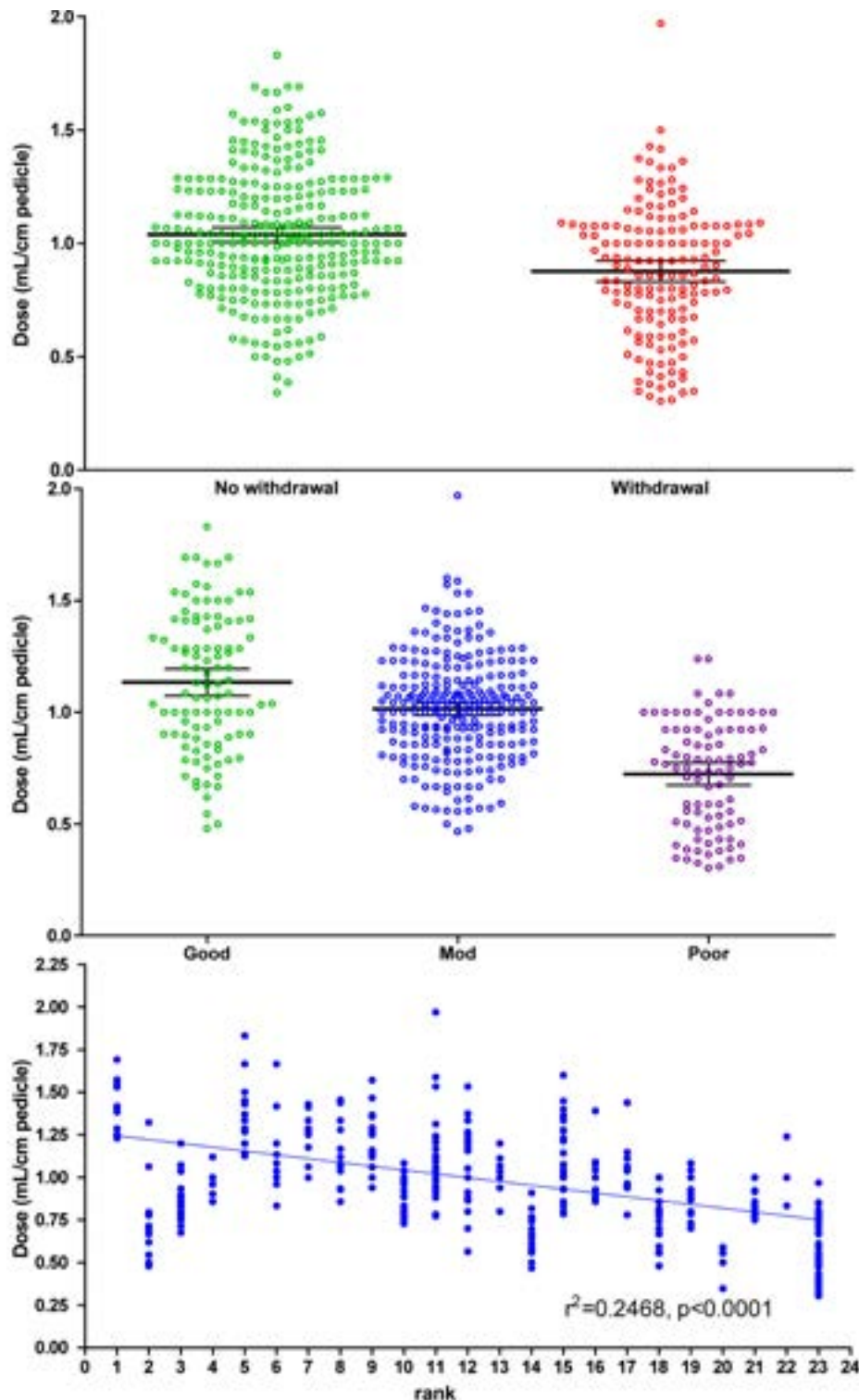


Figure 2.16 “Dose” of lignocaine injected around the antler pedicle by farmers and veterinarians for providing analgesia for velvet removal from adult stags on commercial farms. (Measured in mL per cm of pedicle “Circumference”).

Individual antlers “Doses” are shown as scattered points. The top graph shows the dose rates used for antlers for which the stag did (red) and did not (green) show a withdrawal response during antler cutting. The middle graph shows the dose used by good, moderate and poor operators and the lower graph shows the correlation between operator “Rank” (1=least withdrawal responses to 23= most withdrawal responses) and “Dose”. Lines indicate mean and standard deviation in the upper two graphs and the lower graph also shows the Pearson r^2 coefficient and p value for likelihood that the slope is equal to zero.

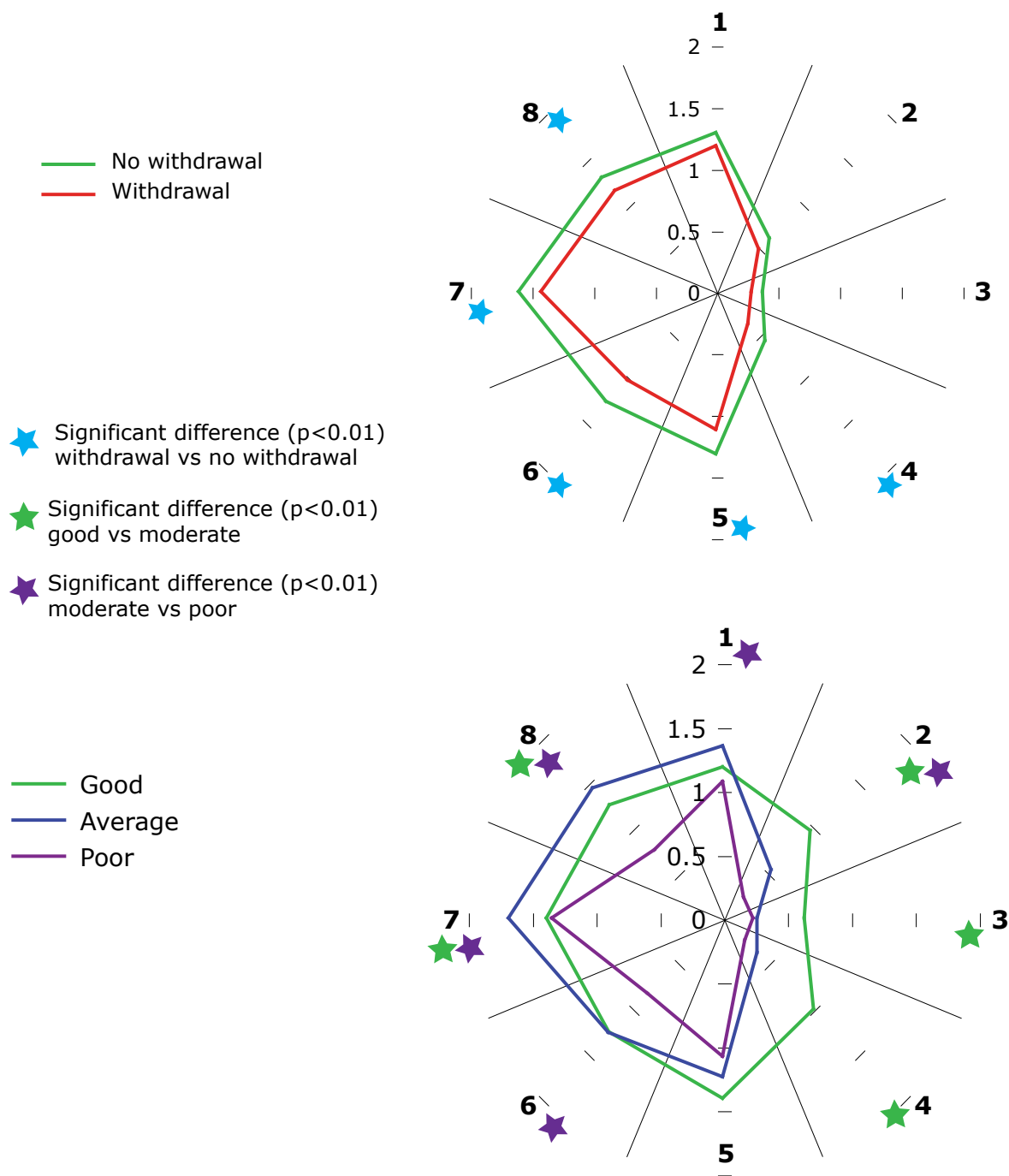


Figure 2.17 Schematic diagram showing the “Dose” (mL per cm “circumference”) of lignocaine injected at each antler “Region” (as shown in Figure 2.4a) by farmers and veterinarians to provide analgesia for velvet antler removal from mature stags on commercial farms.

Upper chart shows antlers for which the stag showed a withdrawal response during cutting (red) versus those that did not (green). Lower chart shows average “dose” used in each “Region” by “good” (green), “moderate” (blue) and “poor” (purple) operators. Significant differences are shown by stars as indicated.

Gaps

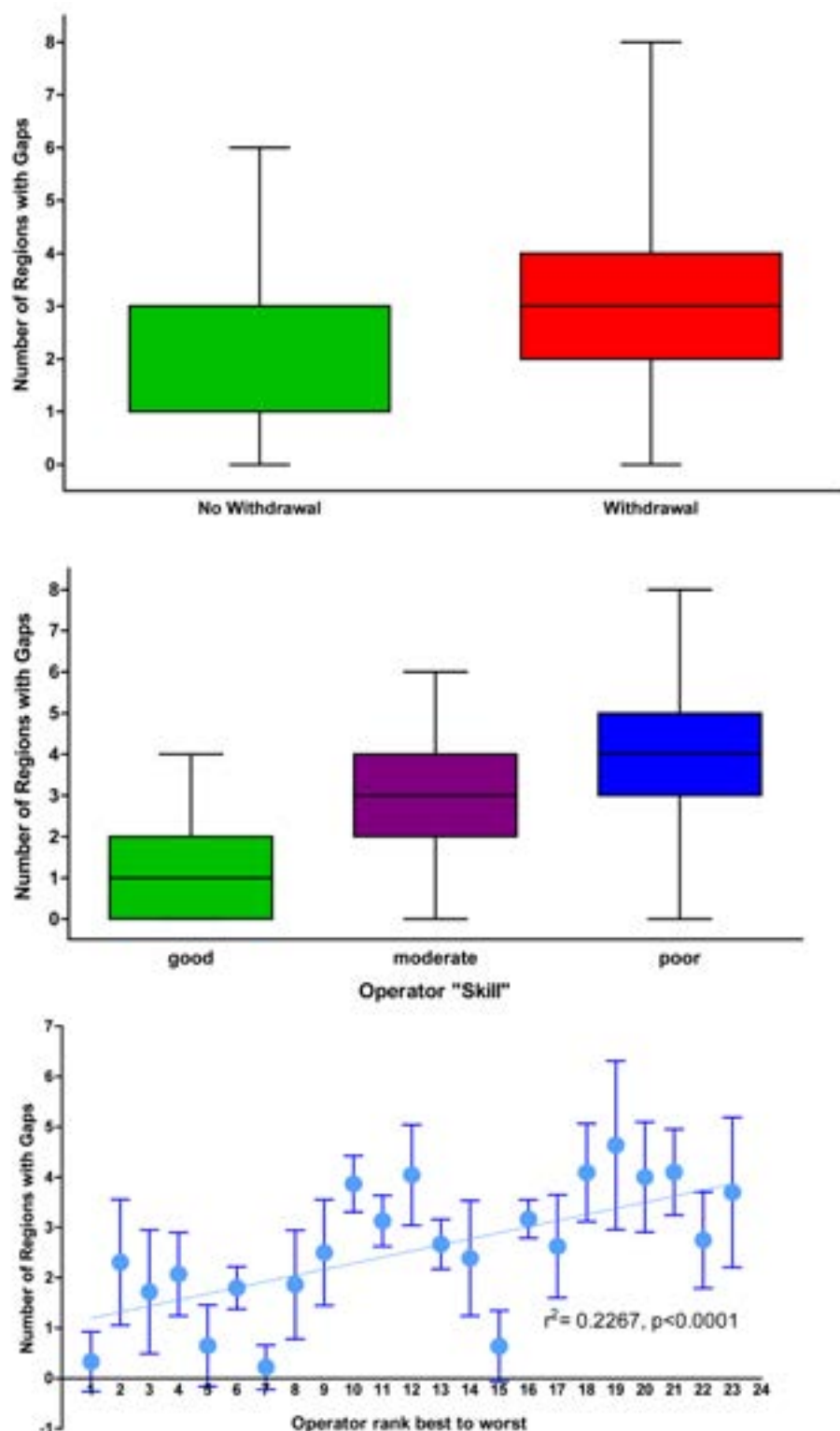
Number of Regions with Gaps (“GapsTOT”)

Visual assessment of lignocaine placement was used to estimate whether a gap in the ring block was present. A “gap” was an area that was predicted to not have lignocaine deposited under the skin across the entire “Region”. Each pedicle “Region” (Figure 2.4a) was classified as either having a “gap” or “no gap”. The maximum number of “gaps” in each “Region” was one. The total number of “gaps” was determined by adding together the number of “Regions” that had a “gap”. This was termed “GapsTOT”. “GapsTOT” ranged from zero (no “gaps”) to eight (i.e. “gaps” in every region).

Figure 2.18 shows the number of “GapsTOT” in the local anaesthetic ring block for individual antlers. Box and Whisker plots of “GapsTOT” from antlers for which the stag did and did not show a withdrawal response during antler cutting are shown in the top panel. In the middle panel the number of “GapsTOT” for antlers removed by “good”, “moderate” and “poor” operators is shown and in the lower panel the number of “GapsTOT” for antlers removed by each operator ranked from best to worse is shown. The latter graph shows the mean and SD of the data.

The mean number of “GapsTOT” was 2.6. Antlers in the “Withdrawal” group had significantly more “GapsTOT” than antlers in the “No Withdrawal” group (mean 3.3 vs 2.5 respectively, $p < 0.0000000001$.) “Good” operators had significantly fewer “GapsTOT” than “moderate” or “poor” operators and “moderate” operators had significantly fewer “GapsTOT” than “poor” operators. When “GapsTOT” was included in logistic regression models it had a significant effect on withdrawal outcome when “Dose” and “Volume” and “Number of injections” were accounted for. The significance of “GapsTOT” was decreased when individuals “gaps” by “Regions” (e.g. “gap 7”) were added to the model. “GapsTOT” was negatively correlated with “Dose” and “Number of injections”.

Figure 2.19 shows a stacked column graph of the proportion of antlers for which the stag did and did not show a withdrawal response during cutting for each number of “gapsTOT”. The number of antlers with each number of “GapsTOT” is shown above each bar in the graph. There were no antlers with seven “GapsTOT” and one antler with eight “GapsTOT”. The antler with eight “GapsTOT” showed a withdrawal response during cutting. For the purpose of graphing and analysis, antlers with six or more “GapsTOT” were grouped. There was a significant correlation between “GapsTOT” and the proportion of antlers for which the stag showed a withdrawal response during antler cutting.



Gaps by Region (“gap 1”, “gap 2”.....“gap 8”)

Figure 2.18 Number of antler “Regions” (Fig 2.4a) that had a “Gap” (“GapsTOT”) in the lignocaine ring block when administered by farmers and veterinarians to provide analgesia for velvet removal from adult stags on commercial farms. Top: Box and Whisker graph of “GapsTOT” for antlers for which the stag did or did not show a withdrawal response during cutting, Centre: Box and whisker of the “GapsTOT” for antlers removed by operators with a “Good (<20% withdrawal), “Moderate” (20-50% withdrawal) and “Poor” (>50% withdrawal) level of “Skill”, Bottom: Mean and SD number of “GapsTOT” for antlers removed by individual operators ranked from best to worst (1=least withdrawal responses, 23= most withdrawal responses).

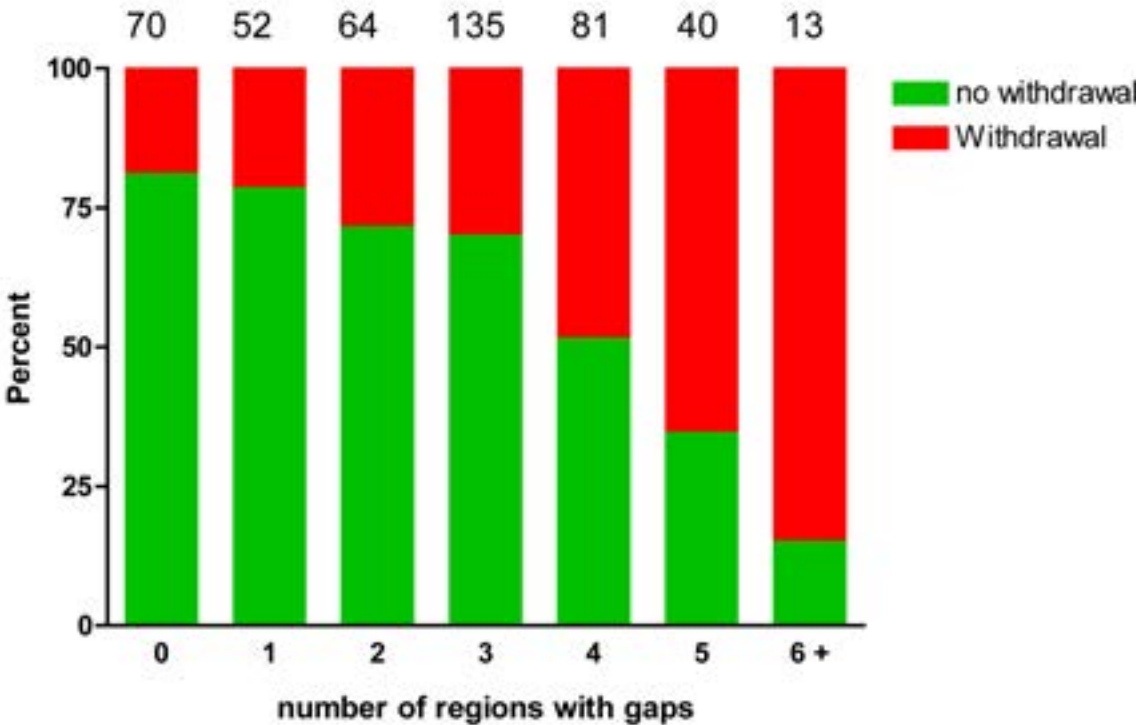


Figure 2.19 Stacked bar graph showing the proportion (percent) of stags that showed a withdrawal response (red) and did not show a withdrawal response (green) for antlers that had 0, 1, 2, 3, 4, 5, and 6 or more “Regions” with “Gaps” (Figure 2.4a and 2.21) in the local anaesthetic ring block around the pedicle. The number of antlers in each group is shown above the bars.

Figure 2.20 shows a schematic diagram of the the number of antlers that had a “gap” in each “Region”. In the top panel antlers for which the stag did and did not show a withdrawal response are shown and in the bottom panel antlers for “good”, “moderate” and “poor” operators are shown.

A significantly higher proportion of antlers in the “Withdrawal” had “gap 1”, “gap 4”, “gap 5”, “gap 6”, “gap 7” and “gap 8” compared to antlers in the “No Withdrawal” group. “Good” operators had a lower proportion of antlers with “gap 1”, “gap 2”, “gap 3”, “gap 4” and “gap 6” than “moderate” operators and “good” operators had fewer “gaps” than “poor” operators in all “Regions” except “Region 7”. “Moderate” operators had a smaller proportion of antlers with “gap 1”, “gap 2”, “gap 5”, “gap 6” and “gap 8” than “poor” operators.

Table 2.22 shows the number and percent of antlers that had a “gap” in each of the pedicle “Regions” and the proportion of antlers that had a “gap” in each “Region” for which the stag also showed a withdrawal response during antler cutting. Also shown is the mean number of “GapsTOT” per antler if a “gap” was present in the given “Region”. For example if there was a “gap” in “Region 1”, there was on average 4.14 “GapsTOT” around the pedicle in total, whereas if there was a “gap” in “Region 3”, the average “GapsTOT” per antler was 3.3. “Gaps” in “Region 1” and “Region 8” tended to be associated with a higher total number “GapsTOT”.

Region 1 (“gap 1”)

55 antlers had a “gap in Region 1”. If an antler had a “gap in Region 1”, 65% of these had a withdrawal response during antler cutting. If there was a “gap in Region 1”, the average “GapsTOT” was 4.15. “gap 1” had a significant effect on the logistic regression.

Region 2 (“gap 2”)

254 antlers (56%) had a “gap in Region 2”. Of the antlers that had a “gap in Region 2”, 37% had a withdrawal response during cutting. If there was a “gap in Region 2”, the average “GapsTOT” was 3.53. “gap 2” did not have a significant effect on the logistic regression.

Region 3 (“gap 3”)

301 antlers (66%) had a “gap in Region 3”. Of the antlers that had a “gap in Region 3”, 37% showed a withdrawal response during cutting. If there was a “Gap in Region 3”, the average “GapsTOT” was 3.35. “Gap in Region 3” did not have a significant effect on the logistic regression.

Region 4 (“gap 4”)

297 antlers (65%) had a “gap in Region 4”. Of the antlers that had a “gap in Region 4”, 39% had a withdrawal response during cutting. If there was a “Gap in Region 4”, the average “GapsTOT” was 3.43. “Gap in Region 4” did not have a significant effect on the logistic regression.

Table 2.22 Number (and percent) of antlers that had a “Gap” in the local anaesthetic ring block in each of the eight “Regions” around the pedicle (Figures 2.4a, 2.21). Of the antlers with a “Gap” in each specified “Region”, the percent that showed a withdrawal response during cutting is shown.

The column on the right shows the mean number of “Regions with Gaps” that was present if the antler had a “gap” in the specified “Region”

Region	Number of antlers with a “gap” in this “Region”	% of all antlers	% withdrawal if a gap in this region	Average number of total “Re- gions with Gaps” if a “gap” was present in this “Region”
None	70	15	23	0
1	55	12	65	4.15
2	254	56	37	3.53
3	301	66	37	3.35
4	297	65	39	3.43
5	82	18	55	4.02
6	116	25	53	4.03
7	30	6.6	70	3.97
8	54	12	69	4.56

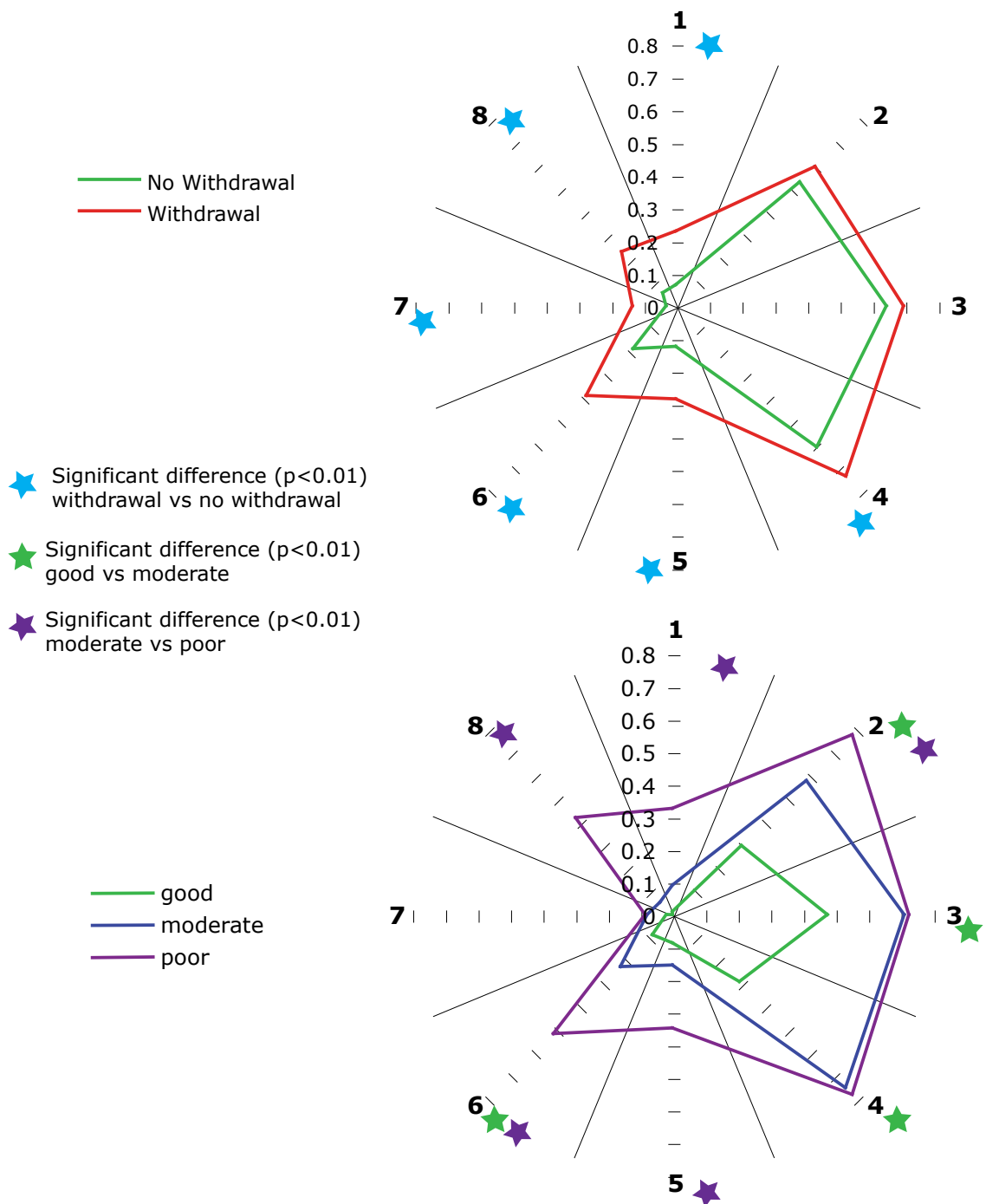


Figure 2.20 Schematic diagram showing the proportion of antlers that had a “gap” in the lignocaine ring block for each of the eight antler “Regions” (as shown in figures 2.4 and 2.21) when administered by farmers and veterinarians for analgesia for antler removal from adult stags on commercial farms.

Upper chart shows the proportion of antlers with a “gap” in each respective “Region” for antlers for which the stag showed a withdrawal response during antler cutting (red) versus antlers that did not (green). The lower chart shows the proportion with a “gap” in each “Region” for “good” (green), “moderate” (blue) and “poor” (purple) operators. Significant differences are indicated by stars as shown.

Region 5 (“gap 5”)

82 antlers (18%) had a “gap in Region 5”. Of the antlers that had a “gap in Region 5”, 55% had a withdrawal response during antler cutting. If there was a “gap in Region 5”, the average “gap-sTOT” was 4.02. “Gap in Region 5” had a significant effect on some logistic regression analyses but not others.

Region 6 (“gap 6”)

116 (25%) antlers had a “gap in Region 6”. Of the antlers that had a “gap in Region 6” 53% showed a withdrawal response during antler cutting. If there was a “gap in Region 6” the average “gap-sTOT” was 4.03. “Gap in Region 6” had a moderately significant on some logistic regression analyses but not others.

Region 7 (“gap 7”)

Thirty antlers (6.6%) had a “gap in Region 7”. Of the antlers that had a “gap in Region 7, 70% showed a withdrawal response during antler cutting. If there was a “gap in Region 7, the average “gap-sTOT” was 3.79. “Gap in Region 7” had a significant effect on the logistic regression model in all analyses.

Region 8 (“gap 8”)

Fifty-four antlers (12%) had a “gap in Region 8”. Of the antlers that had a “gap in Region 8”, 69% showed a withdrawal response during antler cutting. If there was a “gap in Region 8”, the average “gap-sTOT” was 4.56. “Gap in Region 8” had a significant effect on the logistic regression model in all analyses run.

Gaps “lateral side” vs gaps “medial side”

Results in “volume” per antler “Region”, “dose” per antler “Region” and “gaps” per antler “Region” above suggested that there may be a difference between the lateral and medial “Regions” of the antler pedicle. In order to test this further, the antler “Regions” were divided into “lateral” and “medial” sections as shown in Figure 2.21.

Lateral Side Gaps

“Regions” 1, 8, 7, 6 and 5 were combined and the number of “gaps” in these “Regions” were added together. These were designated “lateral side gaps”. Figure 2.22 shows the number of antlers with 0, 1, 2, 3 and 4 or 5 “lateral side gaps” and the percent of these for which the stag showed a withdrawal response during antler cutting. Above each column is the number of antlers that had the corresponding number of “lateral side gaps”. There was a significant trend of increasing withdrawal response with increasing number of “lateral side gaps”. “Lateral side gaps” was used in the final models as a component of “Technique”.

Medial Side Gaps

“Regions” 2, 3, and 4 were combined and the number of “gaps” in these “Regions” added together. These were designated “medial side gaps”. The number of antlers and the percent withdrawal response for antlers that had 0, 1, 2 or 3 “medial side gaps” is shown in the lower panel in Figure 2.22. There was no significant effect of increasing the number of “medial side gaps” on the proportion of antlers for which the stag showed a withdrawal response during antler cutting.

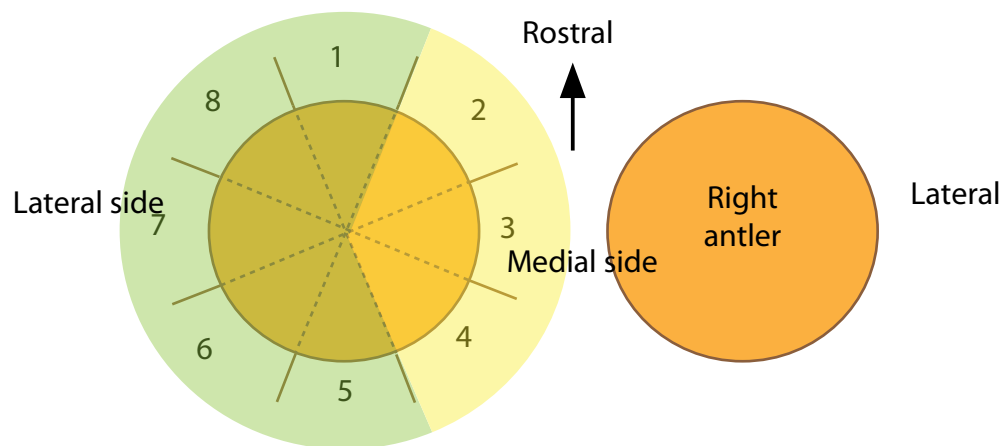


Figure 2.21 Dorsal view of pedicle cross sections showing the “Regions” designated “lateral side” (“Regions” 1, 8, 7, 6 and 5, and “Regions” designated “medial”, (“Regions” 2, 3 and 4) for the purposes of analysing the effect of “gaps” in the lignocaine ring block on the “lateral” or “medial” side of the pedicle on the behavioural response of stags during removal of the same antler

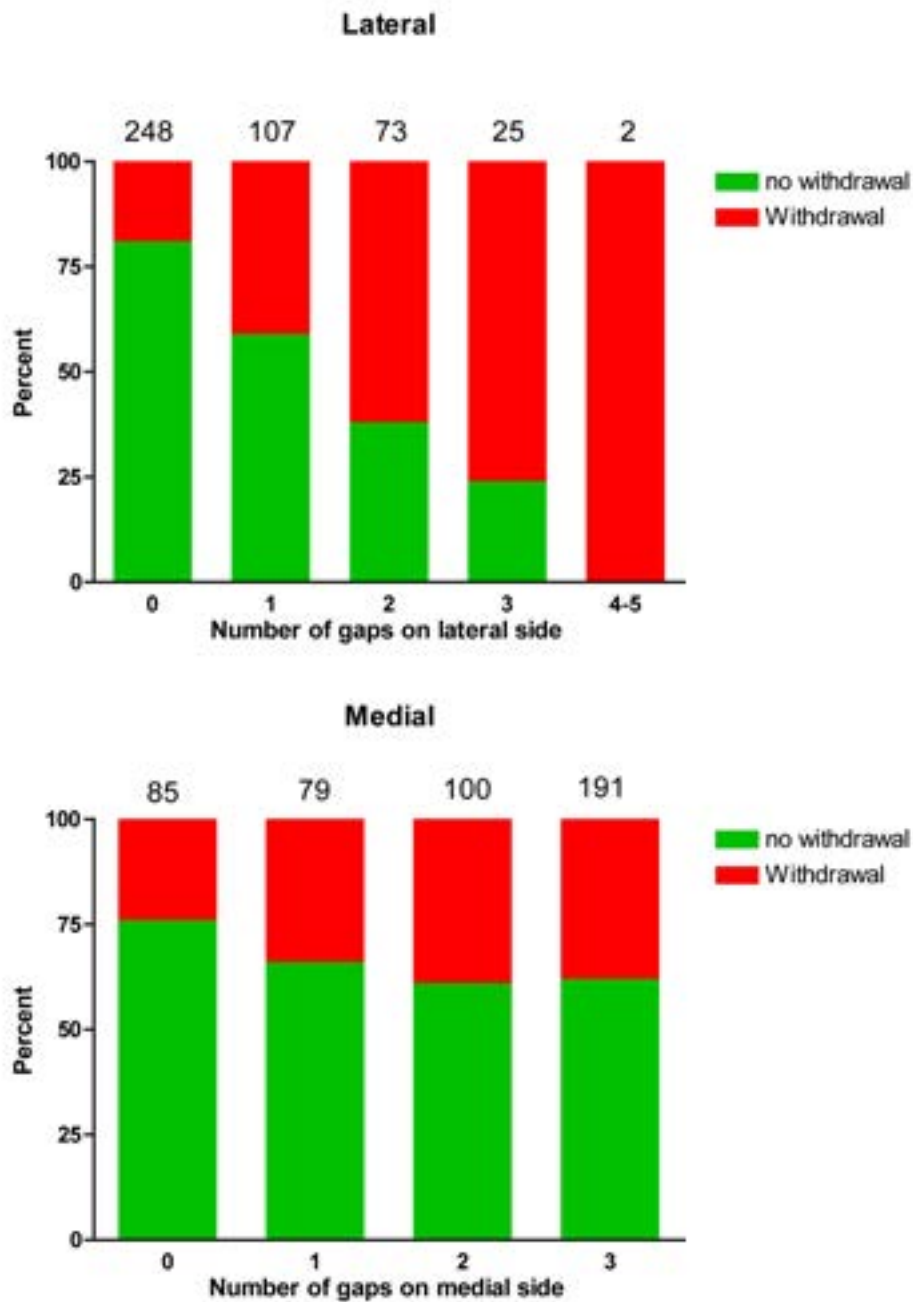


Figure 2.22 Stacked bar charts showing the proportion of antlers for which the stag showed a withdrawal response (red) or did not show a withdrawal response (green) during velvet antler removal for antlers that had different numbers of “Regions with Gaps” on the “lateral” aspect of the antler (top chart) or “medial” aspect of the antler (lower chart) “Regions” are as shown in Fig 2.21. Note that the lateral aspect was divided into 5 “Regions” whereas the medial aspect was divided into 3 “Regions”

Numbers above the bars indicate the number of antlers in each group

Final models

“Technique” Model

Figure 2.23 shows a series of graphs that were produced following sequential addition of significant variables as determined by the analyses above. Addition was made with the most significant factors first and included number of “lateral sections without gaps”, “dose”, “number of injections” and “injection time” in that order. These are shown across the top of the 4 columns of graphs. For each addition, three types of graphs were generated and these are shown in rows of graphs down the page. The top graphs are individual data plots of “Withdrawal” vs “No-Withdrawal” antlers. The second row shows box-plots grouped by operator “Skill” (“good”, “moderate” or “poor”) and the lower graphs are of mean and SD of individual operators ranked from best (fewest withdrawal responses) to worst (most withdrawal responses). At the addition of each variable, groups were separated by a greater and more statistically significant amount and the linearity of the regression line in the operator ranking graphs became more significant.

“Effects” Model

Figure 2.24 shows the “Effects” model. There were many different ways that the final model could be constructed depending on which variables were included and the coefficient used for each. The aim of the model was to separate out antlers for which the stag did or did not show a withdrawal response during antler cutting and describe the combination of factors that are best able to determine the outcome. In this 2-dimensional model an operator “Technique” effect was plotted on the x-axis and a “Stag Factor” effect was plotted on the y axis. Each data point is colour coded for “Withdrawal” (red) or “No-withdrawal” (green).

The following equation was used to combine the significant variables. Coefficients were determined by taking the average coefficient result from the multiple step logistic regression.

“Technique” effect score = (Gap 7 x -1.63) + (Gap 8 x -1.23) + (Number Injections x 0.34) + (Gap 1 x -0.88) + (Gap 6 x -0.7) + (Gap 5 x -0.69) + (Inject time x 0.02) + (“dose” x 1.95)

“Stag Factor” effect score = (Circumference x 0.03) + (Sedation x 1.11) + (Regrowth x 1.20)

This model showed clusters of “Withdrawal” in the upper left part of the graph (low “Technique” score and high “Stag Factor” effect score) and a cluster of “No-Withdrawal” on the right hand side of the graph (high “Technique” score). There was a large group of antlers in the centre of the graph that could not be separated using these equations. These are circled in blue.

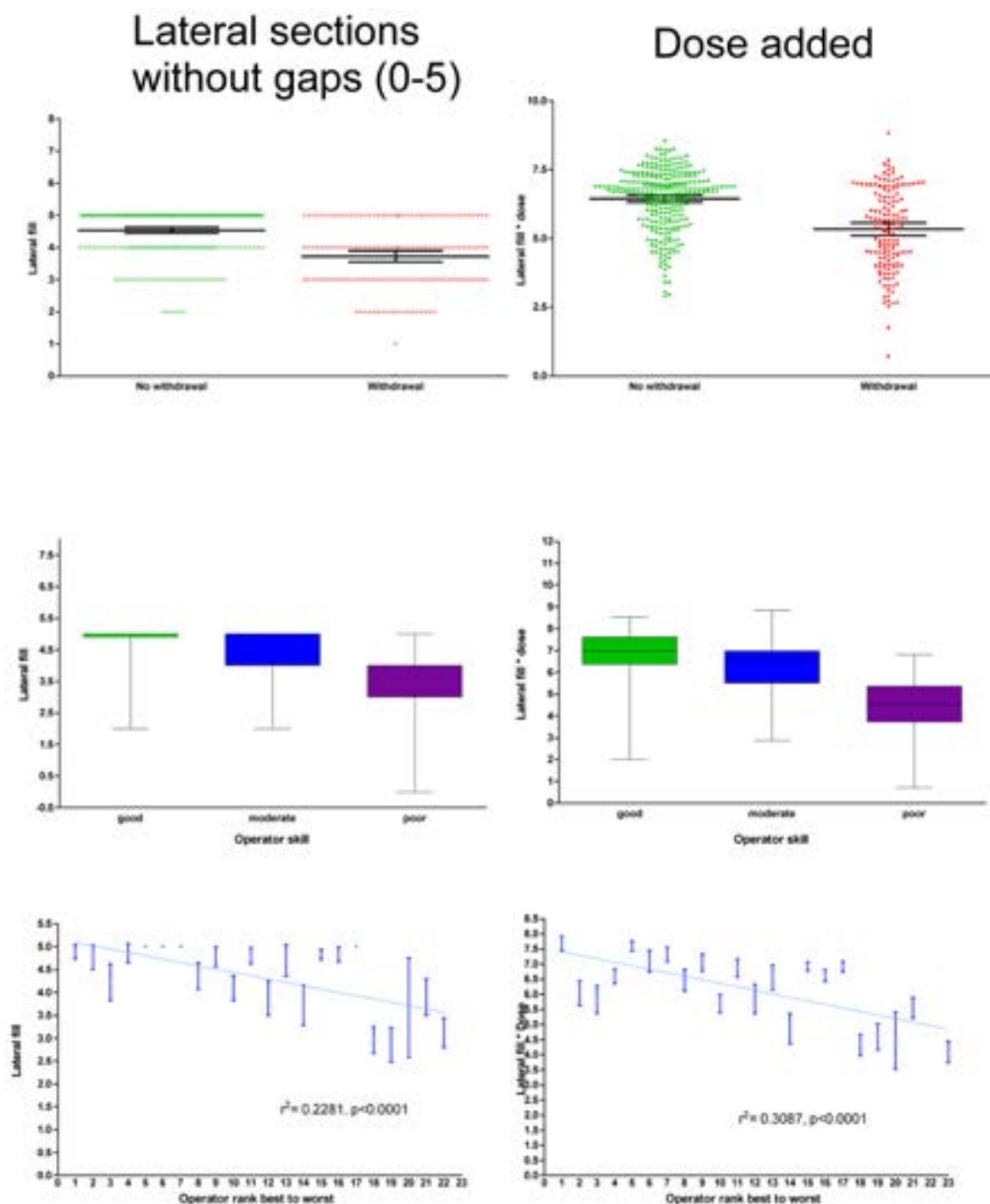


Figure 2.23 “Technique” model showing the effect of adding significant variables related to ring block technique on the difference between outcome groups “Withdrawal vs No Withdrawal” (top row of graphs); “good”, “moderate” and “poor” operator “Skill” (middle row of graphs) and operator “Rank” (1 = fewest withdrawal responses to 23 = most withdrawal responses) in the lower row of graphs.

Factors added include “lateral side gaps” in the ring block, “Dose” of lignocaine use, number of injections (“NumInj”) per antler and time taken to inject local anaesthetic (“InjTime”). In the lower row of graphs, the regression analysis, r^2 and p-value are shown for the correlation between operator “Rank” and “Technique” equation.

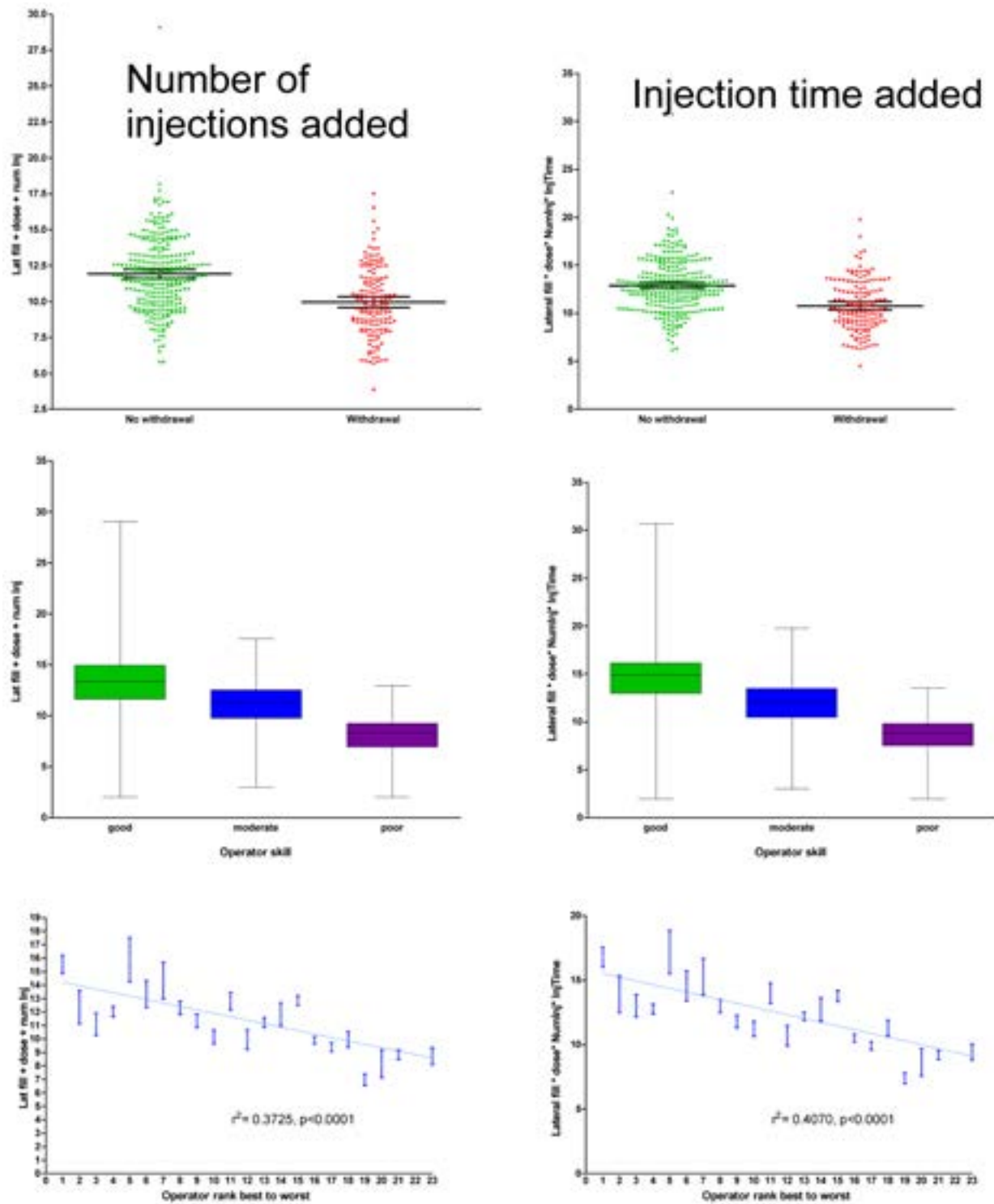


Fig 2.23 continued

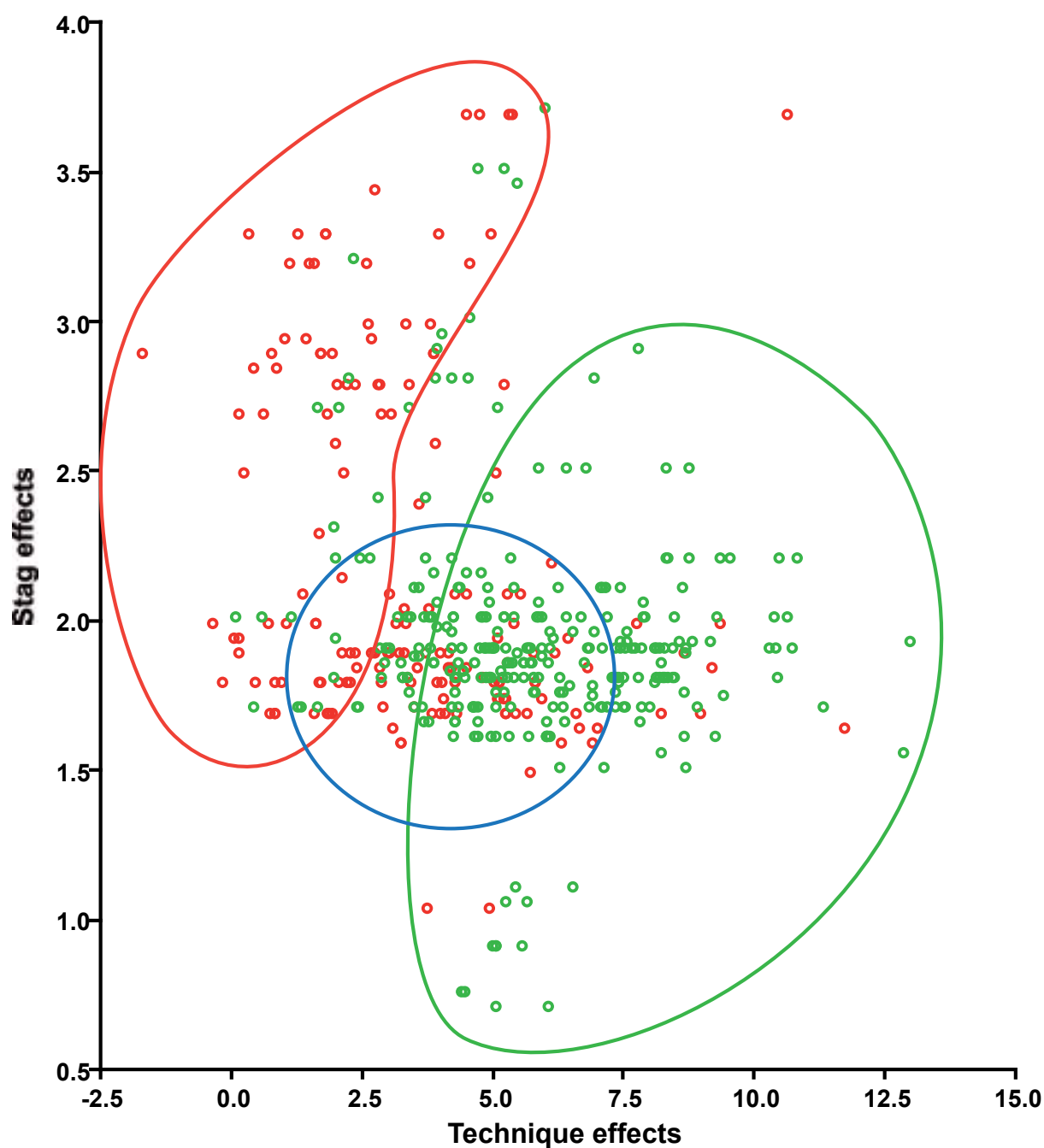


Figure 2.24 “Effects” model investigating aspects of lignocaine injection technique and stag factors to separate antlers for which the stag that did (red) or did not (green) show a withdrawal response during velvet antler cutting when velvet removal was undertaken by farmers and veterinarians on commercial farms.

The red outline indicates the antlers that were mostly in the “Withdrawal” group and the green outlines the antlers that were mostly in the “No Withdrawal” group. Antlers that could not be separated into outcome using this model are outlined in blue. “Stag effects” included “Sedation”, “Regrowth” and “Circumference”. “Technique effects” included “lateral side gaps” in the ring block, “Dose” per antler, “InjectTime” and “NumInj”.

Discussion

This study has used video data analysis to score the behaviour of deer during velvet antler removal and determine the factors that affect the likelihood of a withdrawal response during velvet antler removal. Having gaps in the local anaesthetic ring block was the most significant factor and dose rate, number of injections and injection height also appear to be different between different operators.

Behaviour as an indicator of failed analgesia

The only method used for scoring failure of analgesia in this study was a behavioural response. This meant that any animal that had insufficient analgesia and experienced pain but did not make a physical movement was not identified. The sensitivities of the behaviours used have therefore been overestimated and the number of potential failures can only have been underestimated. A behavioural response that was believed to be induced by cutting the velvet antler with failed analgesia was called a “withdrawal response”. This was mostly to overcome the subjectivity of the term “pain response” since the pain experienced by one individual may be quite different to that experienced by another when the same potentially noxious stimulus is applied. The type and severity of the reaction shown to the same level of noxious stimulus may also vary from one animal to another. It is possible that some animals will react quite violently to a sensation that causes them relatively little pain. If we were able to ask them to grade it on a pain scale say from 1 to 5, it might only be a score 1 or 2 yet they show a dramatic physical response. Another individual may be experiencing quite severe pain (say grade 4 or 5), yet only respond with small physical reactions. Matthews et al (1992) found a linear correlation between electrical stimulation of the velvet antler and the behaviour response severity for individual stags, but also found that there was variation between animals in the minimum intensity required to elicit a response and the threshold at which the maximum level of response was seen. It is not possible to grade the severity of a pain response or partial failure of analgesia without knowing a lot about an individual animal’s pain threshold, general demeanour and likelihood of showing a behavioural response. In this study, only one potential painful episode was observed in each animal. The outcome was either a withdrawal response or no withdrawal response even though some animals may have only conducted a single “movement” during antler cutting while another may have made up to 46 “movements” during cutting. Other authors (Wilson et al, 1999, 2000; Webster et al, 2001, 2003) have tended to use the same approach of grouping animals into a binomial outcome although the cutoff point has been arbitrary. The major difference between this study and previous work is the detailed analysis and grading of behaviours and movements using a more objective, ethogram-type approach. Earlier work has used a functional scoring system, assuming that all movements were related to failure of analgesia. A grading system based on whether the head only was involved or whether the movement also included the neck or whole body has been used. In the ethogram-approach, behaviours were recorded without assuming their function. For example “head arch” was used rather than “pain” or “avoidance” response. The relationship between each of these objectively described behaviours and a “withdrawal” response as a result of failed analgesia was determined after all the data had been recorded. Our work included the behaviour responses described by previous authors as a movement “grade” and found that these were relatively nonspecific for failure of analgesia. This is the first time that specific movements related to the head have been described and analysed.

The behaviours used had low non-specificity, i.e. there were not many false positive results. I assumed that most of the antlers that were graded as a withdrawal response were due to failure of analgesia rather than nonspecific movements. The number of failed analgesia antlers that were not detected may be considerable because the sensitivity of all of the behaviours used was low. Unfortunately there is no way of telling this for certain. While the methods used in this study may be more detailed than those previously described, they have limitations that can only be overcome by combining them with objective methods of detecting failure of analgesia such as physiological changes, electroencephalography or repeated observations in the same animal under different analgesia methods. In the current study these methods were either ethically unacceptable or too expensive.

Statistical analyses of behavioural outcomes

Standard statistical equations were used for determining diagnostic test parameters for the different behaviours recorded in order to assess which behaviours are most suitable indicators of analgesic failure. There are limitations to the method as there is no absolute standard for determining pain. In this case, behaviour was used subjectively to determine the outcome, therefore the results of the sensitivity/specificity analysis only related to responses that involved the stag physically moving. When pain was experienced and the response of the stag was to remain still, it was not detected. Because of this, the relative sensitivity is overestimated. It is also not appropriate to combine behavioural outcomes in an attempt to describe a combination of behaviours that have a higher pain detection sensitivity and /or specificity because it was the combination of these behaviours that was used to determine the outcome in the first place. Naturally, if we included all permutations the sensitivity would arise to 100% because this is what was used to initially determine whether the outcome was likely to be a withdrawal response or not.

Sensitivity was calculated by looking at all the “movements” that occurred during a potential noxious stimulus and determining what proportion of these included the specific behaviour stated. This may be used to predict the proportion of behaviours during potentially noxious stimulus that are likely to include each behaviour. For example we would expect that about 40 percent of the “movements” that occur during a potentially noxious stimulus would include a flinch and that about 46% of “movements” that are graded as a withdrawal response during antler cutting will include a flinch response. It can be seen that no single “behaviour” is a good predictor of whether a noxious stimulus was applied or whether a reaction is likely to be graded as a withdrawal response. Tables 2.8 and 2.9 show that “flinch” was more likely to be seen during a potentially noxious stimulus than when a potentially noxious stimulus was not being applied. The positive diagnostic ratio was 24.5, indicating that a flinch was 25 times more likely to occur during a potentially noxious stimulus than during a time between these events. For the “movements” “grade” slight to 3, and “Look”, it is not relevant to refer to sensitivity and specificity because every “movement” must have included one of these and they are mutually exclusive. If the “movements” were combined then the sensitivity would be 100% and the specificity would be zero since every “movement” in the stimulus group included one of these “grades” and no “movements” in the no stimulus group did not include these behaviours. If each grade is considered independently, the results are meaningless because to determine sensitivity and specificity a binomial outcome is required. In this case we have 5 possible different outcomes. Comparing the relative frequencies of each “movement”

“grade” as above is valid.

There was no correlation between the number of “movements” a stag made before antler cutting and during antler cutting. Stags that moved and jumped around a lot during the waiting periods were not more likely to move during cutting the antler than those that stood quietly during the intervening time periods.

Behaviour recording methods and computer software

The data set could have been more comprehensive, more detailed and flexible to change if behaviour recording software had been used and the behaviour analysis software “Observer” was considered. The cost and complications of using that software, along with perceived inflexibility, did not justify the potential benefit. It is not possible to add or change behaviours after recording has started when using “Observer”. At the time we were unaware of a free and very adaptable software called JWatcher that would work on a Macintosh computer. This software was used in Chapter 3 and would have been useful in this study. The added time and cost of re-entering data into JWatcher was not justified as it was felt that it would add little value to the results that were already achieved by manually entering times and events.

Videos were analysed by a single researcher to maintain consistency in categorising descriptive data. Before analysis, discussion and preliminary viewing of several hours of recordings were undertaken with three experienced animal behaviour researchers, a neurophysiologist and statistician, as well as the researcher, to determine which variables would be recorded and how measurements would be determined for each. This was done because we wanted to avoid using preconceived behaviour outcomes, and detailed behaviour of acute pain due to velvet removal has not previously been described. All previous studies of acute withdrawal behaviour during velvet antler removal in stags have used a grading scale relating to the proportion of the animals’ body involved in the movement and the size of the movement (Matthew et al, 1992; Matthews and Suttie, 2001; Wilson et al, 1999, 2000; Bartels, 2001; Woodbury et al 2001, 2002; Webster et al, 2001, 2003). The number of potential behaviours and descriptions was too great to include all of them in this study so we chose those we considered to be most relevant.

Statistics

This study involved the investigation of multiple variables and their influence on the outcome, being a withdrawal response during velvet antler cutting. Taking video observations from commercial on-farm velvetting procedures allowed for the simultaneous investigation of a number of factors. This was a cost effective and powerful way of identifying the major contributing factors. It would not be feasible or in fact desirable to investigate the effect of such a large range of variables under controlled experimental conditions where numerous permutations would need to be predetermined and investigated. This would require a far greater number of animals. The major disadvantage of the study design used is that many of these variables are confounded with other variables and/or correlated with other variables. It was not possible to fully account for these ef-

fects using statistical methods largely due to the biased distribution of variables with respect to the farm and the operator.

There was a marked difference between operators in the proportion of stags that showed a withdrawal response during antler cutting with the best operator achieving 100% “No Withdrawal” and the worst operator achieving 24% “No Withdrawal”. The statistical methods used aimed to determine which combination of factors caused this difference between operators by exploring the difference between antlers for which the stag did and did not show a withdrawal response and between operators of different “Skill” and “Rank”. Due to the multiple confounding and bias of variables, several different statistical tests were performed. None of the tests were able to find the combination of variables that could predict whether the stag would show a withdrawal response in all cases.

Comparison of different variables between outcomes “Withdrawal” vs “No Withdrawal” and operator “Skill”

There were a number of variables for which significant differences were calculated between antlers for which the stag showed a withdrawal response versus those that did not show a withdrawal response during antler removal. Many of the same variables also showed significant differences between “good”, “moderate” and “poor” operators. As the operator “Skill” (“good”, “moderate”, “poor”) was based on the proportion of withdrawal response it is not surprising that the same variables would be significantly different between these outcome groupings. The variables that showed significant differences between these groupings were likely to be ones that contributed to the overall success of the ring block. The correlation analysis revealed that most of the variables analysed did have some significant correlation even if the correlation coefficient was rarely greater than 0.5. Comparing each variable on an individual variable basis should be done with caution because of these multiple correlations. For example there was a significant difference between outcome groups in “Volume”, “Dose” and “NumInj”. All of these are correlated because they are all related to the amount of lignocaine that was injected. Whether all three of these variables were important in their own right or whether any one of them is more important than the others is difficult to say. Another example is the confounding that was seen between “Dose”, “Circumference” and “Stag Age” with respect the operator “Skill” and “Rank”. There appeared to be a significant difference in “Circumference” and “StagAge” between “Withdrawal” and “No Withdrawal” groups however “good” operators tended to be velvetting stags of lower “StagAge” which is positively correlated with “Circumference” and therefore these stags also had a smaller “Circumference”. There was a negative correlation between “Circumference” and “Dose”, so that “poor” operators were velvetting stags with a larger “Circumference” but also using a lower “Dose”. It is unclear which of these variables are truly important and whether the difference in “StagAge” and “Circumference” was because older stags were velvetted by “poor” operators with less effective technique or whether it was more difficult achieve good analgesia in older stags and therefore those operators velvetting older stags had a lower success rate.

Correlation analysis

Figure 2.5 shows the correlation results for all variables. There were relationships and potential confounding between a number of variables. Few variables were strongly correlated ($r > 0.7$) with other independent variables. None were strongly correlated with the outcome variable (withdrawal or no withdrawal). These data should be interpreted with caution because of the inclusion of both continuous and categorical data and because some data had a Gaussian distribution and others did not. With this in mind, the following correlations were of interest.

“Farm”, “Operator”, “Animal” and “Antler” were all correlated with each other and this is to be expected as each of these were numbered in the order that they were observed and therefore should have a positive correlation. The correlation coefficient was greater than 0.9 between all of these variables. These variables were also moderately correlated with “Tourniquet” ($r = 0.41 - 0.44$), “NeedGauge” ($r = 0.51 - 0.53$), “StagAge” ($r = 0.51 - 0.56$) and “Regrowth” ($r = 0.64 - 0.69$). This was also to be expected as each of these tended to be similar on particular farms. For example the same “Tourniquet” tended to be used for all stags on one farm and a different “Tourniquet” may be used on a different farm. The correlation between these variables reveals bias in the data. These variables are not truly independent as they are not evenly distributed between the farms and operators. This is due to the nature of the data collection method and could not be avoided.

Operator “Skill” and “Rank” were strongly correlated ($r = 0.91$) and this was to be expected as both were determined based on the same criteria, being the proportion of stags that showed a withdrawal response during antler cutting for each operator. Neither “Rank” nor “Skill” were strongly correlated with any other variables but were moderately correlated with some aspects of the injection technique. “Skill” was correlated with “InjectTime” ($r = -0.54$), “NumInj” ($r = -0.53$), “InjHeight” ($r = 0.45$), “Volume” ($r = -0.42$), “Dose” ($r = 0.46$), and gapsTOT ($r = 0.55$). These correlations are of interest and suggest that these aspects of the injection method are important for determining the proportion of stags that show a withdrawal response for each operator. “Skill” and “Rank” were also moderately correlated with “Farm”, “Animal” and “Antler” which is also to be expected as operators of different “Skill” and “Rank” would usually be performing on a single farm. There were some operators that worked on more than one farm and there were also some farms that had more than one operator.

Some other potentially confounded variables that related to the operator included “Injector type”, “NeedLength”, “NeedGauge” and “Tie” which were moderately correlated with each other. This is most likely to reflect the different methods between operators, but consistency within an operator for using a certain set of equipment.

There were also some factors related to the stag that were correlated. “StagAge” was strongly correlated with pedicle “circumference” ($r = 0.79$). These two variables can not be separated as pedicle circumference increases with stag age for several years. It is difficult to separate these variables for further analysis so that it can be determined whether the age of the stag or the size of the pedicle has the greater effect on the withdrawal response outcome. “StagAge” was also strongly correlated with “regrowth” ($r = 0.8$). Stags that were velveted in the regrowth stage of antler growth were more likely to be older stags. This correlation was unfortunate as both of these variables ap-

peared to have an effect but it was difficult to determine the degree of effect that each of these contributed. “Sedation” was moderately correlated with “Regrowth” ($p=0.5$) and “Tie” ($p=0.66$). Stags in regrowth stage of antler were more likely to be sedated which was another unfortunately confounding variable, although further examination of this relationship revealed that once “sedation” was accounted for, “Regrowth” and/or “StagAge” had an even greater effect on the likelihood of a withdrawal response.

There were also some factors relating to the antler and the injection method that were correlated. “InjTime” was moderately correlated with “NumInj” ($r=0.53$). This is logical as the more injections that are administered the longer it is likely to take. “InjTime” was moderately correlated with “TotalTime”, although injection time was a relatively short part of the procedure compared to wait time and “WaitTime” and “InjTime” were not correlated, whereas “WaitTime” and “TotalTime” were strongly correlated ($r=0.79$). “NumInj” was also correlated with “Volume”, “Dose”, dose in “regions” 2,3, and 4 but not “regions” 1, 5, 6, 7 or 8 and with “gapsTOT”. A higher number of injections were related to a higher volume and dose and fewer gaps. “NumInj” was also related to “Pattern” although because “Pattern” contains categorical data it is not as easy to describe this relationship. Logically, a “Pattern” that covers a greater area would require a greater number of injections. This is supported by the finding that “Pattern” was also correlated with dose in “regions” 2, 3 and 4 and “gaps” in “regions” 2,3 and 4. This was most evident in “region 3” and is expected because the highest pattern score was 4 and indicated a complete ring of local anaesthetic around each antler. It is the only “Pattern” that would have covered “Region 3” as the others were either partial ring blocks in a U around the cranial, lateral and caudal part of the pedicle or an arc of less than 75% of the pedicle circumference or an oval around both pedicles together. “Volume” and “Dose” were strongly correlated ($r=0.79$) and this is not surprising as “Dose” was calculated based on “Volume”. It could be expected that this correlation would be stronger considering that both of these variables are continuous data with a normal distribution and directly related to each other. The lower than expected r-value can be explained by the negative correlation between “Dose” and pedicle “circumference” and no correlation between “Volume” and “Circumference”. This suggests that stags tended to be given the same volume regardless of the pedicle size. “Volume” and “Dose” were both positively correlated with dose in “regions” 4,5,6,7 and 8 and negatively correlated with “gaps” in each of the “regions” although this latter correlation was not very strong (all $r \leq -0.41$). There were expected negative correlations between dose in each of the “regions” and the presence of a “gap” in the corresponding “region”.

The order that the antlers were removed, left or right antler and whether it was on the same or opposite side of the crush to the operator were not correlated with any other variable except that the order of removal was strongly correlated with proximity to the operator ($r=0.72$) with the closest antler tending to be removed first. The lack of correlation with all other variables is reassuring as there is no plausible reason that there should be a correlation and therefore this result lends weight to the other data, showing that the strong and moderate correlations found between other variables are likely to be real and significant.

Logistic Regression

Logistic regression should be able to determine the relative significance of different variables when a balanced, unbiased data set is analysed and there is minimal confounding between vari-

ables. Several iterations of logistic regression were attempted with the data set in this study. Logistic regression did highlight some variables of interest but overall was not a highly reliable analysis method for these data. The outcome of the logistic regression analyses varied considerably between different runs depending on the variables that were removed or added at each step. In many instances a judgement had to be made as to which correlated variables were to be removed from the model. This was generally based on the p-value and the odds ratio of each variable after each round of logistic regression. Appendix 2.2 includes two different logistic regression models. One of these is copied into the results section of this chapter. There were differences between these two models that highlight the flaws of this method. For example in the logistic regression included in the results of this chapter, “Dose” did not have a highly significant effect overall. In the other logistic regression shown in Appendix 2.2, “Dose” had the most highly significant effect overall with a high odds ratio range. The main difference between these two analyses was that “dose 1, dose 2.... dose 8” and “gap 1, gap 2..... gap 8” were included in the first analysis whereas these were not included in the second. Having a very large number of variables in the model is likely to decrease the reliability of the results. The logistic regression did show some meaningful outcomes. For example, “Farm” and “Operator” were rarely shown to have a significant effect on the “Withdrawal” outcome. This is despite a clear difference between “Farms” and “Operators” when these variables were looked at independently. The logistic regression showed that the effect of “Farm” and “Operator” were accounted for by aspects of technique and factors relating to the stag rather than each “Farm” or “Operator” *per se*. This finding does add some validity to the other results from the logistic regression.

Multivariate analysis (e.g. Principle Components Analysis) would have been interesting and potentially useful for this data set. In a multivariate analysis factors that are related could be grouped to make a new set of descriptive variables. For example factors relating to the stag (“age”, “sedation”, “pedicle circumference”, “regrowth”) might be combined to have a “stag effect” variable and factors relating to doses (“volume”, “dose”, “number of injections”) could be combined to give a “Amount” variable and perhaps in a third dimension, factors relating to method (“gaps”, “sequential”, “pattern”) might be combined to form a “Method” variable. By creating these new variables in a multivariate analysis it may be possible to better separate and predict the outcome of any particular lignocaine block method. This analysis was not done because there was a high level of confounding in the data which would make the results dubious at best. Very few variables had a high level of correlation so it would take a large number of trials to find the best combination of variables to predict the outcome and even then it is unlikely that the combinations would reduce the variation in the data significantly. Finally, the results of such a multivariate analysis would be difficult to interpret and apply in a practical sense. As the original purpose of this study was to identify factors that improve analgesic efficacy so that they can be applied and incorporated into the velvet removal training programme the time taken to perform such analyses was unlikely to yield any additional useful information. Doing a multivariate analysis on these data would be an interesting statistical exercise if statistical methods were a major goal of the project.

Overall interpretation of the statistics

Exploration of individual variables, correlations between variables and logistic regression were used in combination to gain an impression of the relative significance and contribution of each

variable to the success of the lignocaine ring block. The effect was not truly quantifiable for any of the variables despite logistic regression providing an equation with coefficients for each variable. Those variables that were considered to have the greatest effect were combined in an attempt to separate “Withdrawal” from “No Withdrawal” antlers, “good”, “moderate” and “poor” operators and explain the difference in operator “Rank” using two different models. This method was able to separate some antlers, especially in the “Effects” model where the “Stag Effects” were added. There remained a large number of antlers that could not be separated into “Withdrawal” or “No Withdrawal” based on these models.

Discussion on Independent Variables

Farm (“Farm”)

There was a marked difference between farms in the proportion of antlers for which the stag showed a withdrawal response during removal. The least responses were seen on Farm O, on which only 7% of antlers were considered to have ineffective analgesia, or conversely 93% of antler removals appeared to have sufficient analgesia to prevent a withdrawal response. On Farm P, 73% of antler removals were accompanied by a withdrawal response. When factors relating to local anaesthetic injection technique were accounted for in the logistic regression model, the farm itself did not have a significant effect on the outcome. The difference between farms is most likely to be related to the difference between operators and the technique that they used. As only one operator performed on several different farms, it is not possible to further investigate the possibility of between farm differences. On the farm with the highest number of withdrawal responses, it was observed that the stags tended to be mature in age and mostly in regrowth stage of antler growth. The animals were quiet in the handling facility and moved very little before velvet cutting. The stags that reacted during antler cutting tended to move multiple times with a range of behaviours. The variation between farms and the fact that it appears to be related to technique rather than farm factors *per se* helps to validate the behaviour scoring system used. It shows that when technique is poor, a large proportion of stags will react during antler cutting, whereas when technique is good, very few stags reacted during antler cutting. The statistical model is not very accurate and there are factors relating to farm that may affect the outcome which were not considered in the current study. For example animals on different properties have different demeanours and attitudes relating to their breeding and handling experiences. It is possible that the older quieter stags on Farm P were more likely to show a withdrawal response to a relatively minor stimulus because they were less inhibited by fear and handling. Younger stags that were less well handled may experience a greater level of stress in the handling facility and therefore be less likely to demonstrate a behavioural response to the same level of noxious stimulus.

It is less likely that other farm factors would affect the outcome. Environment, weather, temperature, time of day, nutrition, handling facilities and other farm specific factors were not accounted for in the model. The finding that farm did not have a significant effect suggests that these factors are of little importance.

Operator

Number of years velveting (“YrsVelv”)

The number of years that each operator had been velveting, was not related to outcome success in terms of the proportion of stags that showed a withdrawal response during antler cutting. Operators that had been velveting stags for a long time were not more likely to achieve repeatable analgesia. Similarly operators that were relatively inexperienced did not have a higher rate of failure. This was a surprising finding as it was thought that the more practice an operator had at administering local anaesthetic, the better they would be at it. It appears that good operators are able to modify their technique to ensure that it is effective, whereas “poor” operators continue to use the same standard procedure from animal to animal and from year to year. It would also seem that good operators may be better able to judge a withdrawal reaction in the stags and modify their technique to prevent this from happening, whereas poor operators either neglect to notice a withdrawal response or are unable to modify their technique to prevent them from happening in the future. Poor operators may be unwilling to try a different method from the one that they were originally taught and have become accustomed to over the years.

Operator experience (“Experience”)

By multiplying the number of years velveting by the number of stags velveted per year, the total number of stags velveted was estimated. There was no significant correlation between total number of stags velveted by an operator and “Withdrawal” rate for that operator. There was greater variation in the outcome for operators with relatively little experience. These operators tended to be either considerably worse or considerably better than more experienced operators. The poor performance of some relatively inexperienced operators might be due to lack of practice and ability to judge where the local anaesthetic was placed and the reactions of the stag. The better performance of some of the less experienced operators might be attributable to greater care taken by these individuals as they are still working to establish their technique and are unsure of the expected response thus tending to err on the side of caution. In both cases the result would be dependent on the skill and attitude of the individual operator to the job that they are doing. Caution should be taken when interpreting the results of the operator experience analysis as there was a small number of groups with a large variation and multiple confounding variables.

Equipment

Injector type (“Injector”)

Logistic regression modelling produced varied results with some models suggesting a significant effect of injector type. Injector type is confounded with operator and farm and related to “Dose”, “NumInj” and “Volume”. Logistic regression models were unable to provide a reliable prediction for the effect of injector type. Chi-squared analysis was not valid because there was only one operator that used several of the injector types.

An injector device that delivers a large volume of local anaesthetic (e.g. 5mL vs 2mL vaccination gun) will result in fewer injections for the same “Dose”. The number of injection sites “NumInj” was found to have a significant effect irrespective of “Dose”, therefore it may be concluded that a 5mL injection gun may be less desirable than a 2 mL injection gun at a similar “Dose”. A 1ml injection gun will result in more injections being required. The recommended minimum “NumInj” per antler from the results of this study is one injection per 2cm. Using a 1mL gun, this would result in a “Dose” of only 0.5ml per cm, which is less than the recommended “dose” of 1mL per cm “Circumference” (Wilson et al 1999, 2000). Thus more injections will be required in order to achieve the “Dose”, resulting in inefficiency, the need for a greater number of potentially painful injections and blunting of the needle.

Twenty and 35mL disposable syringes have the advantage that the correct total volume can be drawn up into the syringe at the start of the procedure provided the size of syringe is appropriate to the “Circumference”. Using this type of syringe is likely to be more time consuming as greater care must be taken to ensure delivery of the correct amount at each injection site. However this may result in a more favourable outcome as a longer “InjectionTime” is positively associated with fewer withdrawal responses.

Needle gauge (“NeedGauge”)

Needle gauge was not included in the final models because it was difficult to control for confounding or to explain the effects. Four different gauges of needle were used. A 19 gauge needle was used by one operator in 4 antlers because that operator ran out of 20 gauge needles that were usually used. None of those stags showed a withdrawal response during cutting, but the sample size was too small for comparison. When needle gauge was grouped into 19g and 20g (fine) and 16g and 18g (coarse), antlers blocked with a coarse needle were significantly more likely to result in the stag showing a withdrawal reaction during antler cutting. Withdrawal response rates during cutting were 42% and 30% for coarse and fine needles, respectively. When a table of “NeedGauge” against “Dose” was generated (not shown), animals injected with a coarse needle were more likely to be given a lower “Dose” illustrating that this variable is confounded by other factors related to operator technique. Operators that were classified as “poor” “Skill” were more likely to use a coarse needle.

There was a correlation between “NeedGauge” and “Farm”, “Operator” and “Animal”. The use of different “NeedGauge” was not randomly distributed.

The logistic regression analyses indicated a possible significant effect of “NeedGauge”. It is not possible to draw accurate conclusions from these results because of the high level of confounding. Furthermore, there is no plausible biological reason for the apparent effect. It is likely that better operators chose to use a finer needle but it is unlikely that using a finer needle makes the operator better or improves the placement of local anaesthetic within the tissue planes. Using a finer needle could slow the rate of local anaesthetic flow and allow it to be distributed more evenly as the needle is being inserted but the data from this study is not sufficient to support or refute this. In order to properly assess the affect of needle gauge a controlled study would be required.

Stag Factors

There were factors related to the stag that appeared to affect the outcome. These included “StagAge” “Sedation”, “Regrowth”, “Circumference”, “Farm” and “Operator”. These factors were related to outcome but it was difficult to determine the significance of these because they were confounded by operator effects. Operators with a higher proportion of withdrawal responses tended to be velveting older stags. It is not clear whether the poorer outcome was due to the fact that these operators were velveting older stags or if older stags tended to have a withdrawal response more often because they were velveting using a poorer technique.

Stag age (“StagAge”)

Initial analysis showed that older stags demonstrated a significantly greater proportion of withdrawal responses. Stags in the “Withdrawal” group also had a higher mean “StagAge” than those in the “No Withdrawal” group. If “Sedation” was taken into account, unsedated stags that were 4 years or older were more likely to show a withdrawal response than younger stags i.e. after accounting for “Sedation” the effect of “StagAge” was increased. Logistic regression results predicted that “StagAge” had an effect in some models but not in others, depending on what other variables were included in the model.

There was a greater proportion of older stags in the “poor” operator group. It may be that the “poor” outcome results from the fact that these operators were working with older stags that are more difficult to block. Operators in the “poor” group used a lower “Dose”, less “NumInj”, a lower “InjectSite” and a shorter “InjectTime”, confounding this result. All of these aspects of technique were more significant than “StagAge” in most of the analyses done. It is likely that older stags appear harder to block because a higher proportion of them were blocked with a poor technique compared to younger stags.

Stag age may influence technique. For example an older stag has a larger pedicle diameter. This will mean a greater “Volume” and a greater “NumInj” are required in order to achieve the appropriate “Dose” and “NumInj” per cm. It is also more difficult to inject at a high site on older stags as the coronet becomes very close to the skull and tends to grow wider than the pedicle, leaving very little room to inject local anaesthetic below the tourniquet on the pedicle itself.

Older stags may be more likely to show a behavioural response to pain compared to younger stags. Older stags tend to be quieter and may be less prone to stress induced analgesia or stress induced behavioural responses. Younger stags tend to move more in the handling facility and struggle violently when not being directly handled. These animals may be more likely to hide a pain response or indeed not experience the pain in the same way because their reactions have been overcome with fear. The question of stag age warrants further investigation. During the first eight or so years of the stags life, the antlers increase in size and complexity each year. It is probable that the nerve supply and blood supply also increase in size and complexity with age to supply

the larger antler. The tissue planes around the head and the base of the antler also changes with age, tending to be more difficult to inject as it becomes thickened and more closely adhered to the skin. The factors combined may make it more difficult for local anaesthetic to penetrate to all of the nerves from the antler itself.

Pedicle circumference (“Circumference”)

Pedicle “Circumference” is an indicator of antler size and is related to the age of the stag. The mean “circumference” was larger in antlers in the “Withdrawal” group compared to the “No Withdrawal” group. It is possible that larger pedicles have more nerve fibres crossing them supplying the generally larger antlers. The complexity of the subcutaneous tissues and structures within them including nerves and blood vessels is likely to be increased with larger antlers and pedicles. It was difficult to assess the direct effect of “Circumference” in the current study. Interestingly there was no correlation between “Circumference” and “Volume” whereas there was a negative correlation between “Circumference” and “Dose”. This was a surprising finding and shows that operators do not increase the “Volume” of lignocaine injected when they are velveting older stags with bigger antlers, tending to use the same volume regardless of “Circumference” size. This is contradictory to the recommendation in the velvet removal manual which suggests that the volume of lignocaine used should be determined on a 1mL/cm pedicle circumference basis. These results are further confounded by the fact that the poorer operators were velveting older stags with a larger circumference.

Sedation (“Sedation”)

Table 2.23 shows the number of stags sedated in each age group and the percent withdrawal response for sedated and unsedated stags.

Sedated stags were less likely to show a withdrawal response during antler cutting. A greater proportion of stags 4 years and older were sedated compared to stags 3 years and under. Stags 4 years and older were more likely to show a withdrawal response. When “Sedation” and “StagAge” were examined together, stags that were not sedated were more likely to show a withdrawal response if they were four year or older and this became more significant after accounting for “Sedation”.

For stags 4 years and older 62% showed a withdrawal response if they were not sedated. The effect of “Sedation” was not significant in the logistic regression analysis even when “StagAge” was accounted for in the model when other factors relating to injection method were also included. The disproportionate numbers in the different groups means that statistical comparisons are not valid. The effect of “Sedation” is worth pursuing further because it is estimated that approximately 50% of stags velveted are sedated (Andrew Conway, NVSB pers. comm.). Sedation may improve the efficacy of analgesia or mask the animals behavioural response. The results of this study would have been more meaningful if no stags had been sedated. Even though logistic regression suggested that the difference between sedated and unsedated stags was likely to be due to other factors relating to injection method rather than “Sedation” *per se*. The confounding nature of this

Table 2.23 Number of antlers that were removed by farmers and veterinarians on commercial farms from stags that were sedated or not sedated in different age groups (2years old, 3 years old and 4 years and older) and the percent withdrawal response during antler cutting for each group.

Stag Age	Sedated		Not Sedated	
	Number of antlers	% Withdrawal	Number of antlers	% Withdrawal
2 years	6	0 %	194	29 %
3 years	8	25 %	121	31 %
4 + years	48	25 %	78	62 %

factor is important and would be better avoided in the future.

Stage of antler growth - First cut versus regrowth (“Regrowth”)

Stags in regrowth antler showed more withdrawal responses than stags in primary growth antler. All regrowth cut was on stags over 2 years of age and the results would therefore be influenced by the same factors that affected the difference seen with “StagAge”. The operator with the worst success rate was cutting regrowth which also biased these results. “Regrowth” is also likely to be confounded by “Sedation” in this study as this was related to “StagAge” as discussed above. It would have been preferable to avoid this confounding factor of “Regrowth” velvet in the current study.

Regression analysis found no significant effect of “Regrowth” in most models that included important aspects of operator technique. These results indicate that regrowth velvet is well innervated and at least as sensitive to pain as primary growth velvet. Further controlled study into regrowth is warranted as at least one of the participants in this study commented that they found regrowth more difficult to block than primary growth on the same animal. Some other operators did not share this experience.

Wilson et al (2000) found that regrowth velvet is likely to be as sensitive as first cut.

Head-Tied (“Tie”)

Some farmers elected to tie the stags head down with ropes or harnesses during antler cutting. It was still possible to observe the range of behavioural responses in stags with their heads tied down, especially the lower “grades” of “movement”. Whole body struggle (“grade 3”) and “Look” were most likely to be reduced by tying the head. On one farm an assistant would hold the stag’s head down by leaning on the nose with his bodyweight. The stag was still able to throw the assistant off and lift its head up high during cutting. “Tie” did not appear to have a significant effect on to proportion of antlers for which the stag showed a withdrawal response during cutting in any of the comparisons done.

Timing Factors

Tourniquet time (“TournTime”)

“TournTime” was not seen as relevant to the outcome because there was no plausible explanation for how the time taken to apply a tourniquet could affect a withdrawal response outcome. It was another confounding variable that was better removed from the modelling equations. Tourniquet application time is considered in further detail in the Chapter 4. The results from the tourniquet data gathered in the current study (tourniquet type, application time and haemostasis) will form the preliminary observations in the project on lignocaine residues and tourniquets.

Injection time (“InjectTime”)

There was a significant difference between all outcome groups with respect to mean “InjectTime”. Antlers in the “Withdrawal” group had, on average a shorter “InjectTime” than those in the “No Withdrawal” group. Operators with a “good” “Skill” level were more likely to take longer to inject local anaesthetic than those with a “poor” “Skill” level and there was a significant negative correlation between “InjectTime” and operator “Rank” (best to worst). When “InjectTime” was added to the logistic regression model, the significance of the effect was variable. Longer “InjectTime” was associated with a greater “NumInj” and fewer “gapsTOT”. “InjectTime” was included in the model because there was a significant or near significant effect in most analyses. The coefficient weighting was small at 0.02. “InjectTime” may be longer for successful blocks and better operators because it takes longer to inject a larger number of sites and it also takes longer to make certain not to leave any “gaps” in the ring block. “InjectTime” alone does not accurately predict the outcome. There were some antlers that took a long time to inject but still had a withdrawal response. There were a larger number of antlers that took only a short time to inject but did not show a withdrawal response. The worst operators tended to have a shorter and less variable “InjectTime” suggesting that these operators use a more consistent method between stags. Operator 18 did not fit the trend as that operator had a consistently short “InjectTime” and a relatively good success rate (75% “No-Withdrawal”).

Wait time (“WaitTime”)

“WaitTime” was the time between injecting local anaesthetic and cutting the antler. It might be expected that the longer the operator waits, the more chance the local anaesthetic has to diffuse through tissue planes and reach the nerves from the antlers. The “WaitTime” recommended in the NVSB velvet removal manual is 2 minutes. Within the range of “WaitTimes” observed in this study there was no effect of this variable on the outcome and the antler with the shortest “WaitTime” of 1.08 minutes was not associated with a withdrawal response from the stag during removal. 52/429 antlers had a “wait time” less than 2 minutes. It would seem that “WaitTime”’s shorter than two minutes may also be effective. This result is similar to previous studies. Morrow and Matthews (1998) found no effect of wait time on outcome. Bartels et al, (2001) showed that some stags ceased to respond to electrical stimulation of the antler from 15 seconds after injection of lignocaine. Two minutes may not be long enough for some individual animals. Bartels et

al (2001) reported that the longest time a stag took to stop responding to electrical stimulation after injection of lignocaine was 195 seconds (3 mins, 15 secs). Wilson et al (1999) found that after 2 minutes 3/18 stags responded to electrical stimulation of the antler and after 4 minutes in the same group of stags, 1/18 responded. The lack of statistical significance is probably due to the observation that only a small proportion of stags appear to benefit from a longer wait time. The dilemma is whether it is more important to wait longer to ensure that all stags have been given sufficient time resulting in most animals having a longer wait time than required or to observe a shorter wait time with the risk that a small number of stags may not have had sufficient time. If the latter approach is taken, it will be important to test for analgesia carefully and allow further time for any stag that responds to the first nick test. Most operators observed a "WaitTime" considerably longer than two minutes with the average being more than three minutes. Most operators will do some other task during the waiting time such as moving deer through pens, labelling antlers or weighing and recording antlers.

Cut time ("CutTime")

"CutTime" was a highly significant factor. This is reasonable to expect as if a stag moves during cutting it slows the progress considerably. While "CutTime" had a significant effect in the logistic regression model it was removed early from the modelling because "CutTime" is likely to be an outcome of withdrawal response rather than a contributing factor. "CutTime" had such a large effect on the logistic regression model that it would mask the effects of other variables relating to technique. "CutTime" was significantly longer in antlers that had a withdrawal response, yet there was no clear effect of operator "Rank". Operators that were more successful did not consistently have a shorter "CutTime". "CutTime" was skewed to the right with some antlers taking a very long time to cut. These tended to be with poorer operators but some of the antlers that had a withdrawal response for "good" operators also had an unusually long "CutTime" supporting the hypothesis that "CutTime" should be treated as an outcome of "Withdrawal" rather than an independent variable.

Total time ("TotalTime")

"TotalTime" was significantly correlated with "WaitTime". "TotalTime" did not have a significant effect on the outcome overall. It is interesting to look at the relationship between the different times. Operators with a higher proportion of stags showing a withdrawal responses tended to have a shorter "InjectTime" and a longer "WaitTime". Only "InjectTime" had an effect on withdrawal response. As "InjectTime" ranged from seven to 104 seconds and "WaitTime" ranged from 65 to 747 seconds it seems logical to conclude that the time saved by using a shorter "InjectTime" was likely to be lost in a longer "WaitTime". Antlers with a shorter "InjectTime" were more likely to result in a withdrawal response and were therefore likely to take longer to cut. The longest "CutTime" was 353 seconds. So by taking an extra 20 seconds to inject the local anaesthetic and waiting a shorter time (2 minutes instead of 3 minutes) it is more likely that a withdrawal response will not be seen and the "TotalTime" per antler will be shorter. In fact, there was a significant difference in "TotalTime" between antlers for which the stag demonstrated a withdrawal response (6.2 ± 2.7 minutes) versus those that did not (5.6 ± 2.0 minutes) $p=0.016$. It is predicted that this

time could be shortened further but was confounded by different operators undertaking various activities during the intervening periods as described in “WaitTime” above.

Antler order (“Order”), proximity (“Proximity”) and side (“Left/Right”)

Antler “Order”, “Proximity” and “Left/Right” did not affect whether there was a withdrawal response or not. There was a relationship between “Order” and “Left/Right”. More (61%) antlers were removed with the operator standing on the left side of the animal. There was also a relationship between “Order” and “Proximity”. In 86% of cases, the antler closest to the operator was removed first however only 53% of first antler removals were left antlers. This suggests that of the 14% of antlers removed from the opposite side of the crush first, the majority of these were right antlers with the operator standing on the left hand side of the crush.

The finding that antler “Order”, “Proximity” and “Left/Right” was not correlated with other variables is reassuring when looking at associations or correlations between other variables. It shows that the random variation in the data is sufficient to conclude that an apparent relationship is indeed meaningful.

Factors relating to method of injection

Number of injections (“NumInj”)

The number of injection sites per antler appears to affect the outcome. “Good” operators use a greater “NumInj” than “poor” operators and antlers in the “Withdrawal” group had a lower “NumInj” than those in the “No Withdrawal” group.

Figure 2.25 shows the relationship between “NumInj” and “InjectTime” and Figure 2.26 shows the relationship between “NumInj” and “Volume”. In these scatterplots, the data points have been colour coded according to whether the stag did (red) or did not (green) show a withdrawal response during removal of the antler. The more injections that were given, the longer it took and the greater the “Volume” injected. The correlations between “NumInj”, “InjectTime” and “Volume” were significant but not very high. Some operators were able to give a large “NumInj” very quickly whereas some other operators took a long time to administer a small “NumInj”. This is probably related to stag temperament during injection and operator speed. Of the antlers that took more than 40 seconds to inject, very few had a withdrawal response regardless of the “NumInj” and of the antlers that had 9 or more injection sites, very few had a withdrawal response regardless of “InjectTime”. This result suggests that both of these factors have an important and fairly independent effect on the outcome and thus both can justifiably be included in the final “Technique” model.

“Volume” was related to the “NumInj” but again the correlation was not very strong. It is surprising that there is such a poor correlation as it would be expected that the more injections were

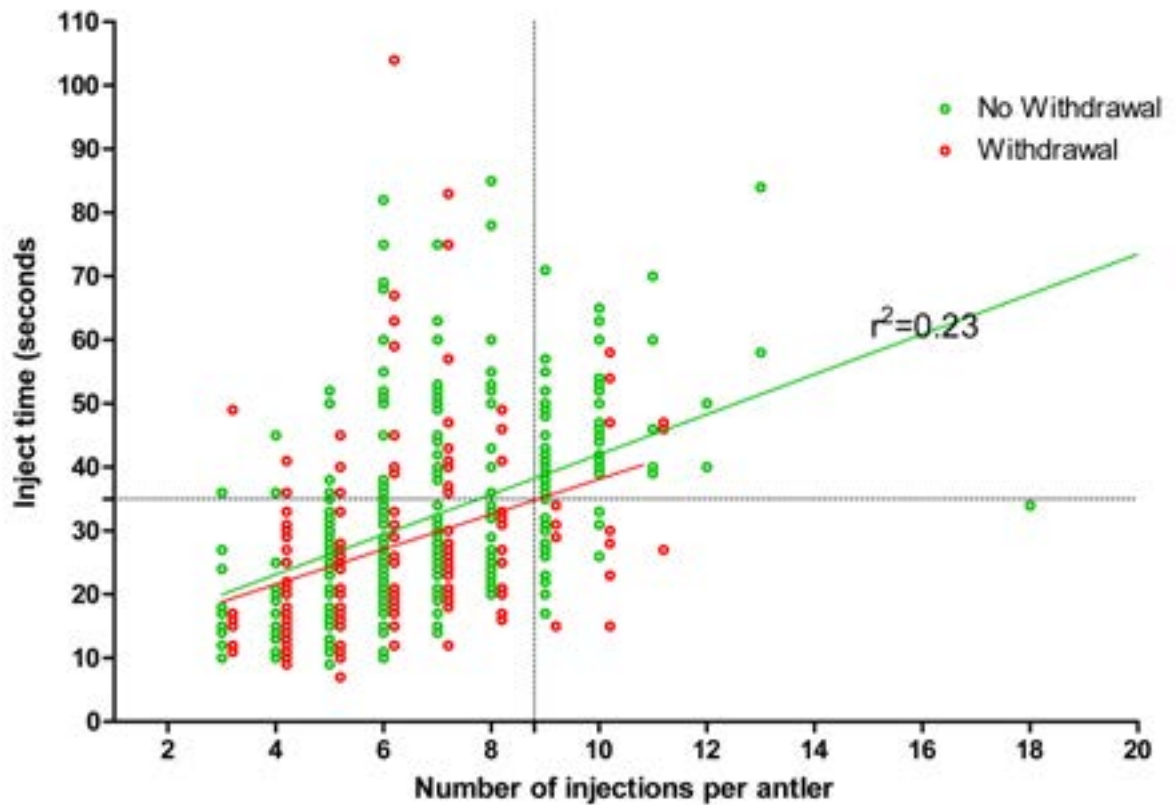


Figure 2.25 Scatterplot of the number of injections per antler versus time to inject around each antler when local anaesthetic was injected in a ring block for velvet antler removal. The pearson r^2 correlation coefficient is shown.

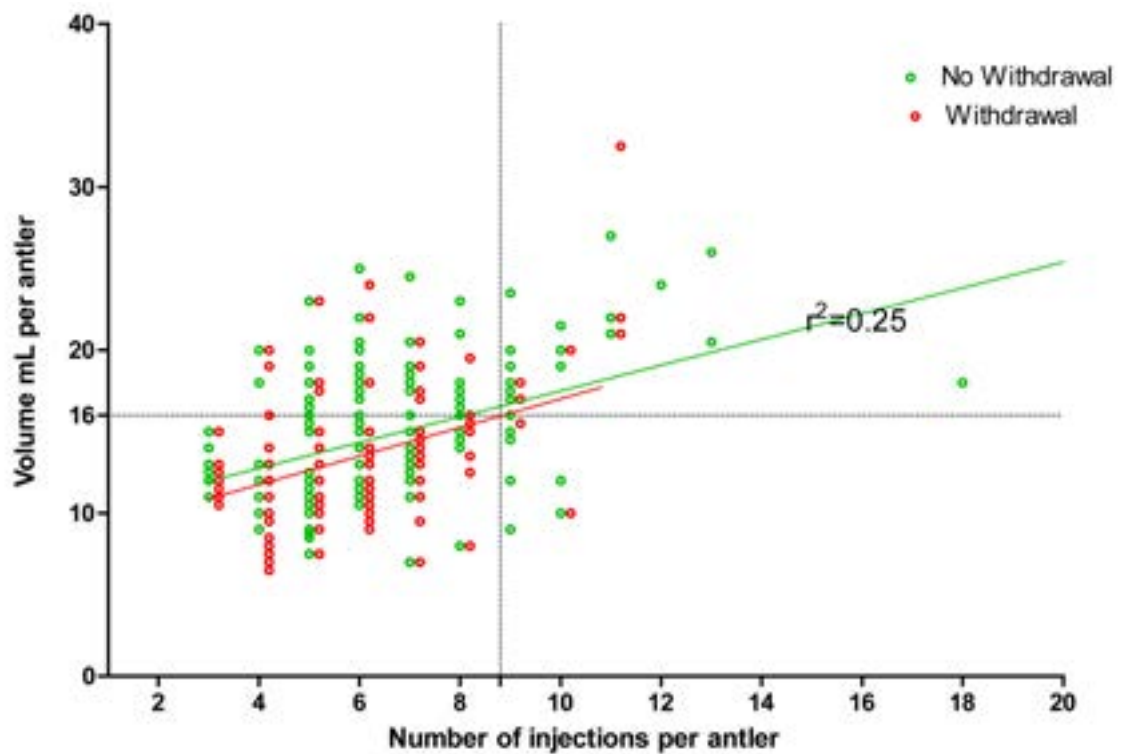


Figure 2.26 Scatterplot of the number of injections per antler versus total volume injected around each antler when local anaesthetic was injected in a ring block for velvet antler removal. The pearson r^2 correlation coefficient is shown. Variation around the regression line is due to differences in volume per injection.

delivered, the higher the total “Volume” would be. The variation in this relationship can be accounted for by the different delivery systems used and the volume injected at each site. Some operators used an injection gun set at one millilitre and others used an injection gun set at 5 millilitres. Some operators would deliver only part of the loaded dose at one site or would deliver multiple doses at the same site. Few antlers that were given at least 16mL “Volume” were associated with a withdrawal response regardless of the “NumInj” and few antlers that were given more than 9 “NumInj” showed a withdrawal response regardless of the “Volume” used.

The relationship between the “NumInj” and “Dose” was even less well correlated than “NumInj” and “Volume”. This is because the “NumInj” and “Volume” were not correlated with “StagAge” or “Circumference” of the pedicle. When “Dose” was calculated by dividing the “Volume” by “Circumference”, there was greater random variation in the data.

It is difficult to say if the apparent effect of the “NumInj” is in itself an important factor or if the “Volume” injected and “InjectTime” are more significant.

Volume (“Volume”) and dose (“Dose”)

Injecting a high “Volume” or a high “Dose” in itself did not guarantee to prevent a withdrawal response. The highest “Volume” injected was 32.5mL and that stag showed a withdrawal response during the removal of that antler. There was considerable overlap in the “Volume” and “Dose” of local anaesthetic used between antlers in the “Withdrawal” and “No Withdrawal” groups with some “No Withdrawal” antlers having been injected with a surprisingly low “Volume”.

“Volume” was strongly correlated with “Dose” and this was expected because “Dose” was calculated based on the “Volume” and “Circumference”. There was no correlation between “Volume” and “Circumference” or “StagAge” and there was a negative correlation between “Dose” and “Circumference” and “StagAge”. This is surprising and suggests that operators are more likely to use a consistent “Volume” regardless of the size of the antlers which would reflect in a decreasing “Dose” for larger sized antlers. It was considered that “Dose” was a better proxy for the amount of local anaesthetic used than “Volume” although neither of these variables had a strong effect in the logistic regression analysis when “gaps” for different “Regions” were included in the analysis.

In order to further investigate this relationship the “Dose” was divided into 6 different dose-range groups and the proportion of antler removals during which a withdrawal response was demonstrated in each dose range was calculated. This is shown in Table 2.24 and Figure 2.27. At the upper and lower limits of “Dose” there was a clear difference in withdrawal response. Of the 24 antlers that were injected with a “Dose” less than 0.5mL/cm, 19 (79%) were associated with a withdrawal response and 5 were not. At the other end of the spectrum, of the 22 antlers that were injected with 1.5mL/cm or more, 20 were not associated with a withdrawal response and 2 (9%) were associated with a withdrawal response. There is a relatively flat region from 0.75 to 1.25 mL/cm and on either side of this there appears to be a strong trend that increasing “Dose” decreases the likelihood of a withdrawal response. The “Dose” rates required to drop the withdrawal response to low levels are higher than the 1.0mL/cm currently recommended. As discussed previously it is probable that other factors also influence the outcome and while a high “Dose” in

Table 2.24 Number of antlers injected by farmers and veterinarians on commercial farms with different dose (mL/cm pedicle circumference) ranges of lignocaine and the proportion of these for which the stag showed a withdrawal response during velvet antler cutting.

Dose range	Number of antlers	% Withdrawal
< 0.5	24	79 %
0.5 to 0.74	60	46 %
0.75 to 0.99	131	34%
1.00 to 1.24	152	34%
1.25 to 1.49	58	19%
1.5 +	22	9 %

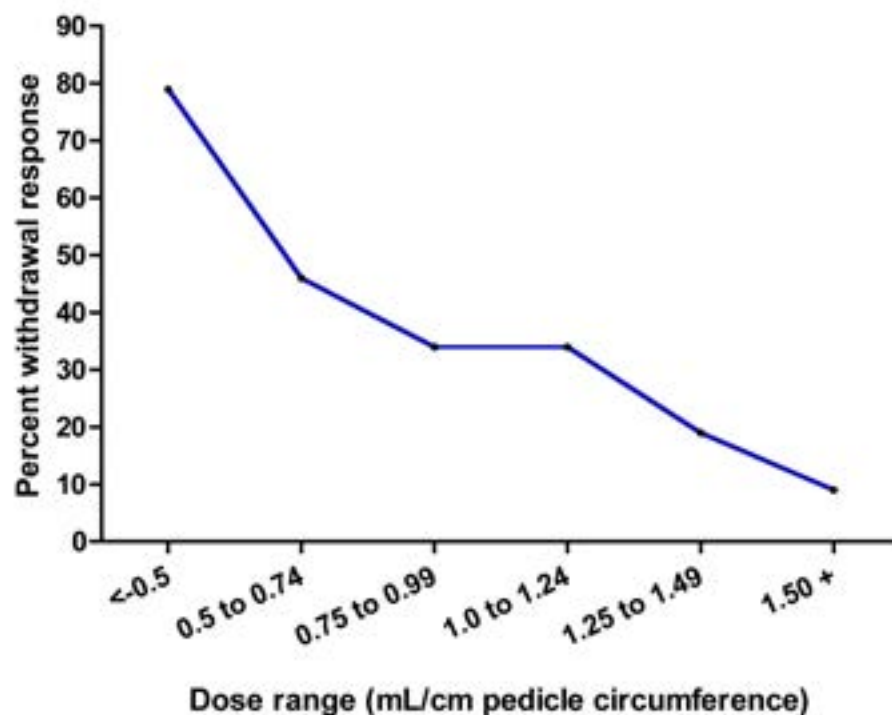


Figure 2.27 Trend between “dose” and proportion of antlers for which the stag showed a withdrawal response within each 0.25mL/cm pedicle circumference grouping. Proportion withdrawal response was the number of antlers for which the stag showed a withdrawal response divided by the total number of antlers injected with that “dose” range.

itself may increase the chances of a successful block, if other factors are used in combination with a moderate “Dose” the outcome may be equally successful.

Dose per region (“dose 1, dose 2..... dose 8”)

In general antlers for which the stag did not show a withdrawal response tended to have a higher “dose per region” around the entire antler but this was only significant for “dose 4”, “dose 5”, “dose 6”, “dose 7” and “dose 8”. This is in contrast to the results based on operator “skill” as “good” operators injected significantly higher “dose 2”, “dose 3” and “dose 4”, but injected a lower “dose” on the “lateral side” of the antler, notably “dose 7” and “dose 8” when compared to “moderate” operators. “Poor” operators tending to inject a lower “dose per region” around the antler but with the most remarkable feature being a lower “dose 8”. A gap in this “Region” (“gap 8”) was one of the most significant factors in the logistic regression analyses. It appears that “good” operators tend to distribute the local anaesthetic more evenly and consistently around the pedicle, particularly on the “medial side” of the pedicle. The need for injecting local anaesthetic on the “medial side” of the antler is contradicted by the observation that there was no difference in “dose per region” in these regions between antlers in the “Withdrawal” and “No Withdrawal” groups.

Gaps (“gapsTOT”)

“Gaps” in the anaesthetic ring block were determined by watching the videos and marking out on the diagram in Figure 2.2 the approximate area covered by each of the injections of lignocaine. This is a rather crude way of determining the distribution of the local anaesthetic but it is the only means readily available in practice. It may be useful to inject dye or labelled lignocaine to get a more accurate indication of lignocaine distribution but that would require slaughter of the animals and dissection of the injection sites.

Overall “gapsTOT” had a very significant effect on the likelihood of a stag showing a withdrawal response during antler cutting. In the logistic regression models “gapsTOT” accounted for much of the effect related to “Dose”, “Volume” and “NumInj”. This suggests that the ring block may still be successful when a lower “Dose”, “Volume” or “NumInj” was used, provided there are no “gapsTOT”. Woodbury and Haigh (1996) suggested that a ring block of overlapping subcutaneous injections around the entire base of the pedicle should be used in all cases and the results here support that recommendation along with those of other authors (Matthews et al, 1992; Wilson et al, 1999, 2000). Wilson et al (1999, 2000) has tended to focus more on the “Dose” of local anaesthetic and height of the ring block with respect to the antler rather than overlapping injection sites. This appears to be the first experiment investigating the effects of gaps in the ring block.

It may not be necessary to have overlapping injection sites around the entire antler pedicle as suggested by Woodbury et al (1996). There was little difference between 0 and 1 “gapsTOT” or between 2 and 3 “gapsTOT” in the proportion of antlers for which the stag showed a withdrawal response. The lack of clear trend with a smaller number of “gapsTOT” is possibly because when

there are only a small number of “gapsTOT” they are more likely to be on the “medial side” of the antler. It would be unwise to assume that having one or two “gapsTOT” is fine without considering the location of those “gaps”. Limited studies on antler innervation (Adams. 1979; Woodbury and Haigh, 1996) have shown that sensory innervation is via the infratrochlear and zygomaticotemporal nerves and possibly branches of the auriculopalpebral nerve. Dissection of these nerves showed that the larger branches travel up the lateral, cranial and caudolateral aspects of the head below the pedicle. The distribution and course of smaller branches closer to the pedicle and antler have not been described. It is not known, for example, what the route of the nerves supplying the medial aspect of the antler is. The results of this study suggest that small nerve branches course to the medial aspect of the antler distal to the site of the ring block.

Good operators left very few “gaps” in the ring block around the whole antler except in “Region 3”. “Moderate” operators were also careful to leave few “gaps” on the “lateral side” of the antler but were much more likely to leave “gaps” on the “medial side”, having a higher proportion of antlers with “gap 2”, “gap 3” and “gap 4”. “Poor” operators had a high proportion of “gaps” on the “medial side” of the antler and were more likely to have a “gap 2” than “moderate” operators. The most striking aspect of “poor” operators was the high proportion of “gap 8”, “gap 6” and “gap 1”. “gap 8” was the most significant factor in one of the logistic regression analyses and “gap 1” was also highly significant. It may be that simply filling these “gaps” on the “lateral side” with extra injections of local anaesthetic will greatly improve the outcome for the “poor” group of operators. It may also be the case that “good” operators could reduce the amount of local anaesthetic used on the “medial side” of the antler and still maintain good efficacy at applying the ring block. This suggestion is further supported by the observation (Figure 2.22) that leaving 1, 2 or 3 “gaps” on the “medial side” of the antler made little difference to the proportion of stags that had a withdrawal response, but leaving just one gap on the “lateral side” increased the percent of antlers that had a withdrawal response significantly.

Final Models

The final modelling stage was an attempt to combine significant variables into an overall “Technique” score that would better separate antlers for which the stag showed a withdrawal response during antler cutting from those that were not associated with a withdrawal response. It is evident that there are a large number of confounding variables that have an effect on the likelihood of a withdrawal response during antler cutting and some of these were either not thought of in this study or not able to be easily measured. The nature of the data collection method meant that the data were not randomly distributed across all variables. For example it was not possible to separate “stag effects” and “Technique” effects because “poor” operators were more likely to be velvetting older stags but also used a smaller “Volume” and “Dose” and had more “gapsTOT”.

The “Technique” model (Figure 2.23) looked at technique only and sequentially added each of four important variables. These were: number of lateral regions without “gaps”, “Dose”, “NumInj” and “InjectTime”. At each step the antlers in the “Withdrawal” and “No Withdrawal” groups were plotted along with the scores for “good”, “moderate” and “poor” operators and the scores for operators “Ranked” from best to worst. At each addition, the differences between these groups be-

came greater and more significant (decreasing p-value). The correlation between operator “Rank” and “Technique” score improved with each addition. This model was able to add together some factors that, in combination, better explained the differences between groups than each factor on its own, however it did not manage to separate antlers in the “Withdrawal” group from those in “No Withdrawal” group very effectively. This leads to the conclusion that the model was not very good either because the wrong variables were included or there are other factors that influence the outcome that are not accounted for.

The “Effects” model (Figure 2.24) adds another set of variables to try to better separate out antlers in the “Withdrawal” and “No Withdrawal” groups. This was termed “stag effect” and included “Circumference”, “Regrowth” and “Sedation”. In this model there appears to be better separation of the “Withdrawal” and “No Withdrawal” groups with the top left of the graph (poor “Technique”, high stag score) mostly consisting of “Withdrawal” outcomes and the lower right (high “Technique” score, low stag score) mostly consisting of “No Withdrawal” outcomes. Unfortunately there were not many antlers in the high “Technique”/high stag score region. There remained a large cluster of antlers around the middle of the graph that could not be separated on withdrawal response so again this model was not particularly effective and could not be reliably used to predict whether a withdrawal response is likely to occur.

The final model shown in Figure 2.28 is a schematic diagram of the interactions or likely interactions between the variables that have been discussed in this study. This figure shows that there is considerable overlap between factors and multiple interconnections. Thus preventing a withdrawal response during velvet antler cutting is a complex combination of multiple factors some of which can not be controlled. The biggest influence is likely to come from the technique that the operator is using to inject local anaesthetic and using a good technique has a higher chance of a successful block.

None of the statistical models were able to completely separate the groups. In the “Technique” model (Figure 2.23), there was some improvement in goodness of fit when different factors were added but the improvement overall was marginal. This is probably because the combination of important aspects of technique could not be fully described using the variables recorded in this model. It is also probable that one variable may negate the need for another. For example if injections are placed sequentially with overlapping blebs (i.e. no “gaps”), this may be more important than injection “Volume” *per se* and so a lower “Volume” may be used when the good operator is comfortable that they have injected enough local anaesthetic to effect a good block with no “gaps”. There may not be a prescriptive method that will guarantee the outcome in every event. Every stag and every situation may be slightly different and it may only be the experience and judgement of the “good” operator that is able to adjust to each individual occasion. A prescriptive method does not allow scope for such adaption. So the operator that gives the same “NumInj” in the same sites every time may have a lower overall success rate even though they are more consistent and may score highly on the significant factors in combination (“NumInj”, “Dose”, “Volume”, “Inject-Time”).

When operators were grouped according to “Skill” and averaged, the differences appeared to be more marked than those seen in individual operator ranking. The interesting outcome here was

that when more variables were added to the “Technique” model, the “poor” operators become more tightly grouped, whereas the “good” operators tended to maintain quite a large spread. As discussed above this may be due to the ability of the “good” operator to adapt to the requirements for each individual animal. In judging the placement of local anaesthetic they may use more or less as they deem it to be required. A higher or lower injection site may be chosen depending on the anatomy of the area and the “InjectTime” may vary depending on the circumstances. For the operators with a poorer outcome, they may be more consistent, using a similar “InjectTime”, injection sites, “NumInj” and “Volume” for each animal. Thus when these factors are combined, the spread becomes smaller, whereas the greater variation in the “good” operators tends to make this spread become larger.

It was more difficult to separate “Withdrawal” vs “No Withdrawal” antlers using the models in this study compared to separating operators based on “Skill” or “Rank”. This is surprising as this analysis looked at variables independent of the operator. In this case, when variables were added to the “Technique” model, the “No-withdrawal” group become slightly more spread out and the “Withdrawal” group become slightly less spread relative to each other. This effect was not as great as in the operators grouped by “Skill” model. This result shows that even when the important variables are combined, it does not guarantee a successful outcome, although the chances of success are greater and on the other hand when the combination of variables are lacking, it does not guarantee a failure, although the chances of failure are greater.

These results create a paradoxical debate on the best method for auditing operators to ensure that the animals are being provided with full and effective analgesia. On the one hand, judging the operator based on the “technique” used may be useful because operators with a good “technique” are more likely to achieve analgesia and operators with a poor technique are more likely to have withdrawal responses. If the technique is being audited, then only a small number of velvet removal occasions need to be observed. The results of this study show that even when a good “technique” was used, some stags still showed a withdrawal response and even when a poor “technique” was used, some stags did not demonstrate a withdrawal response. If the “technique” is being assessed then it would not matter what the outcome was in terms of stag behaviour. There are other factors besides those identified in this study and included in “technique effects” that may influenced the success rate. “Good”, “moderate” and “poor” operators could be separated quite well but not all of this was explained by “technique”. “Good” operators, by definition had fewer withdrawal responses. If the audit focused on an outcome based assessment, without concern for the “technique” used to achieve that outcome, then it may take into account other factors that this study did not identify but that influence the outcome. Using an outcome based assessment, a greater number of animals would be required because of the variation in response between animals i.e. if one animal out of three demonstrated a withdrawal response despite a good “technique” being used, that operator would have a success rate of 66%. If another operator used a poor technique, yet two of the three animals still did not demonstrate a withdrawal response despite this, then that operator would also have a success rate of 66% even though the two have quite different levels of “technique”. This may be influenced further by the age of the stag and stage of growth of the velvet. It is probable that greater consistency would be achieved by auditing the “technique” used rather than the behavioural outcome of the stag even though all of the factors that constitute a good “technique” and other factors that might influence the behavioural response of the animal

have not been fully identified.

Conclusions

The local anaesthetic, lignocaine, is effective at preventing a withdrawal response during velvet antler removal only if it reaches the axons of all the sensory nerve fibres travelling from the antler. Achieving successful analgesia depends on a complex combination of correlated and inter-related factors, the most important of which is the technique used to inject local anaesthetic. A good technique will increase the chances of success because local anaesthetic is more likely to be placed close to all the nerves.

Good technique should include having no “gaps” along the caudal, lateral and cranial aspects of the pedicle, using a high number of injection sites, using a high dose of local anaesthetic and taking more than 30 seconds to inject around each antler. Older stags, especially those in regrowth may be more difficult to provide with effective analgesia so extra attention should be paid to ensure good injection technique is used in these animals.

Successful analgesia can only be readily determined in the field by the absence of a behavioural withdrawal response during antler removal. Judging a movement during antler removal as a withdrawal response should be based on a combination of behaviours as no single behaviour had a high sensitivity and specificity for failure of analgesia. The best indicator of a withdrawal response was a short sharp flinch type “behaviour” at the time of antler removal and this occurred in 46 percent of withdrawal responses.

Chapter 3

NaturOTM ring

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Introduction

This chapter evaluates the effects of compression analgesia using the *NaturO*TM ring for velvet removal from yearling stags. Removal of velvet from yearling stags is an industry dilemma whereby farmers and industry organisations have to balance the needs of welfare during transport, analgesia during velvet removal, drug residues in meat or velvet and the need for timely sale of young stags to slaughter. *NaturO*TM rings are rubber bands that are placed on the pedicle of the antler for one hour prior to antler removal and it is proposed that analgesia is achieved by nerve compression. They have provided many solutions but their efficacy and the potential pain induced during use has not been fully assessed. These issues, along with the methods used in this study are discussed in Chapters 1.2 and 1.3. The following four experiments were conducted using electroencephalography and behaviour (discussed in Chapter 1.2.2) separately to evaluate the response of rising one-year-old deer to application of *NaturO*TM rings and subsequent velvet antler removal. The aim was to assess if *NaturO*TM ring application was painful and if the analgesia they provided was comparable to a local anaesthetic block.

3.1 Effects of a *NaturO*TM ring or lignocaine ring block on the EEG response to cutting the velvet antler of one-year-old red deer under general anaesthesia.

Introduction

Electroencephalography was used by Johnson et al (2005) to assess the efficacy of compression analgesia using a high pressure compression band in wapiti deer. This study used the method described by Johnson et al to investigate the efficacy of *NaturO*TM rings in yearling red deer. The use and advantages of EEG are discussed in Chapter 1.2.2.

Material and methods

Study design

Controlled prospective experiment.

Animals

Thirty eight one-year-old male red deer weighing 85kg (sd 8 kg) were studied at the Massey University Deer Research Unit (DRU). These were a combination of 21 animals bred and reared on the DRU and a cohort of 17 stags bought in from a commercial farm and managed on the DRU. Of these, 25 were anaesthetised once and 13 were anaesthetised twice.

At each occasion, only one antler was removed, being the longest of the two antlers at the time of the experiment.

Treatments

Deer were randomly assigned to one of three treatment groups (N=17 antlers per treatment) prior to the experiment:

- Local anaesthetic (Lignocaine)
- *NaturO*TM ring (*NaturO*)
- Control (no analgesia) (Control)

Lignocaine

Lignocaine hydrochloride (20mg/mL) ("Nopaine 2%", Phoenix Pharm) was placed subcutaneously in a complete ring around the base of the antler using a 20 gauge 1 inch needle attached to a vaccination gun and 500 mL flexipack of lignocaine. Approximately 15mL total volume was

administered over eight to 10 overlapping sites around the pedicle 10 to 30 minutes prior to antler removal.

NaturO™ ring

A standard NaturO™ Ring (AgResearch) was applied using the tied method (Farmer Velvet Ant-



Figure 3.1.1 Anaesthetic procedure and monitoring equipment set-up for EEG recording during velvet antler removal. Top left : Placement of 20 gauge catheter in the facial vein. Top right, intubation standing in the crush by manually advancing the hand and tube through the dental arcade, lower: Maintenance on halothane in oxygen with an out-of-circuit rebreathing system.

Figure 3.1.2 Recording sheet for details of anaesthetic drugs and monitoring during general anaesthesia for recording the electroencephalogram during velvet antler cutting for deer that had been previously treated with either a lignocaine ring block, a NaturO™ ring or no treatment.

assist intubation.

After injection of the induction agent, a size 14 or 16mm ID endotracheal tube was placed in the trachea by manually advancing the hand and tube through the oropharynx and manipulating the larynx. The cuff was inflated and the deer transported on a sling to an air bed elevated on a table. The ET tube was connected to a circle anaesthetic system and halothane in oxygen delivered via a precision vaporiser (Fluotec III) at an initial fresh gas flow rate of 1.8 ± 0.7 litres per minute and halothane concentration of $3.5 \pm 0.8\%$. This was reduced to approximately 0.8 ± 0.25 L/min fresh gas flow rate and a vaporiser setting of $2.5 \pm 0.5\%$ halothane at the time of recording.

End tidal halothane concentration and expired carbon dioxide were measured with an anaesthetic gas analyser (Hewlett Packard). Somatic movements, palpebral reflex, end tidal halothane, respiratory rate and character, heart rate and character, pulse quality and EEG waveform were used to assess anaesthetic depth. An electrocardiogram (ECG) was recorded continuously using a two lead base (right jugular groove) - apex (left thorax) configuration.

At the completion of EEG data collection, the stag was disconnected from the anaesthetic machine and transported to a recovery pen. 3mg/kg of ketoprofen (Keloprofen 10%, Phoenix Pharm

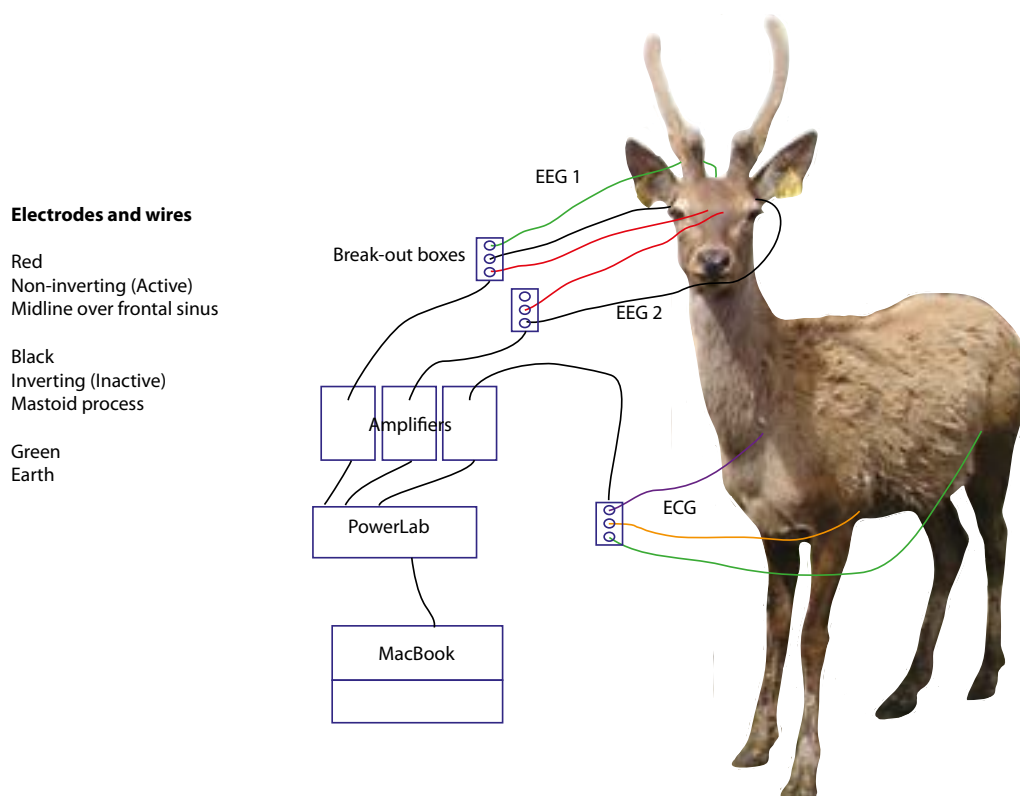


Figure 3.1.3 Set-up of EEG recording equipment and leads for measuring the electroencephalogram of deer during velvet removal after treatment with either a lignocaine ring block, a NaturO™ ring or no treatment. Two channels of EEG were recorded, one from each cerebral hemisphere. A single earth lead was connected with one of these montages only. Each montage was connected to an individual breakout box which then fed into an amplifier. These were connected to a Powerlab and then to the laptop computer for recording.

Distributors Ltd) was administered IV for post-surgical analgesia to all animals.

EEG recording equipment and procedure.

Figure 3.1.3 shows the two channels of EEG recording and one of ECG. Figure 3.1.4 shows a close up of the positioning of the electrodes. Two channels of EEG data were recorded simultaneously from the left and right cerebral cortices.

The EEG electrodes were arranged in a bipolar montage: that is, each signal is measured between two separate electrodes. For each channel, an inactive (non-inverting) stainless steel needle elec-

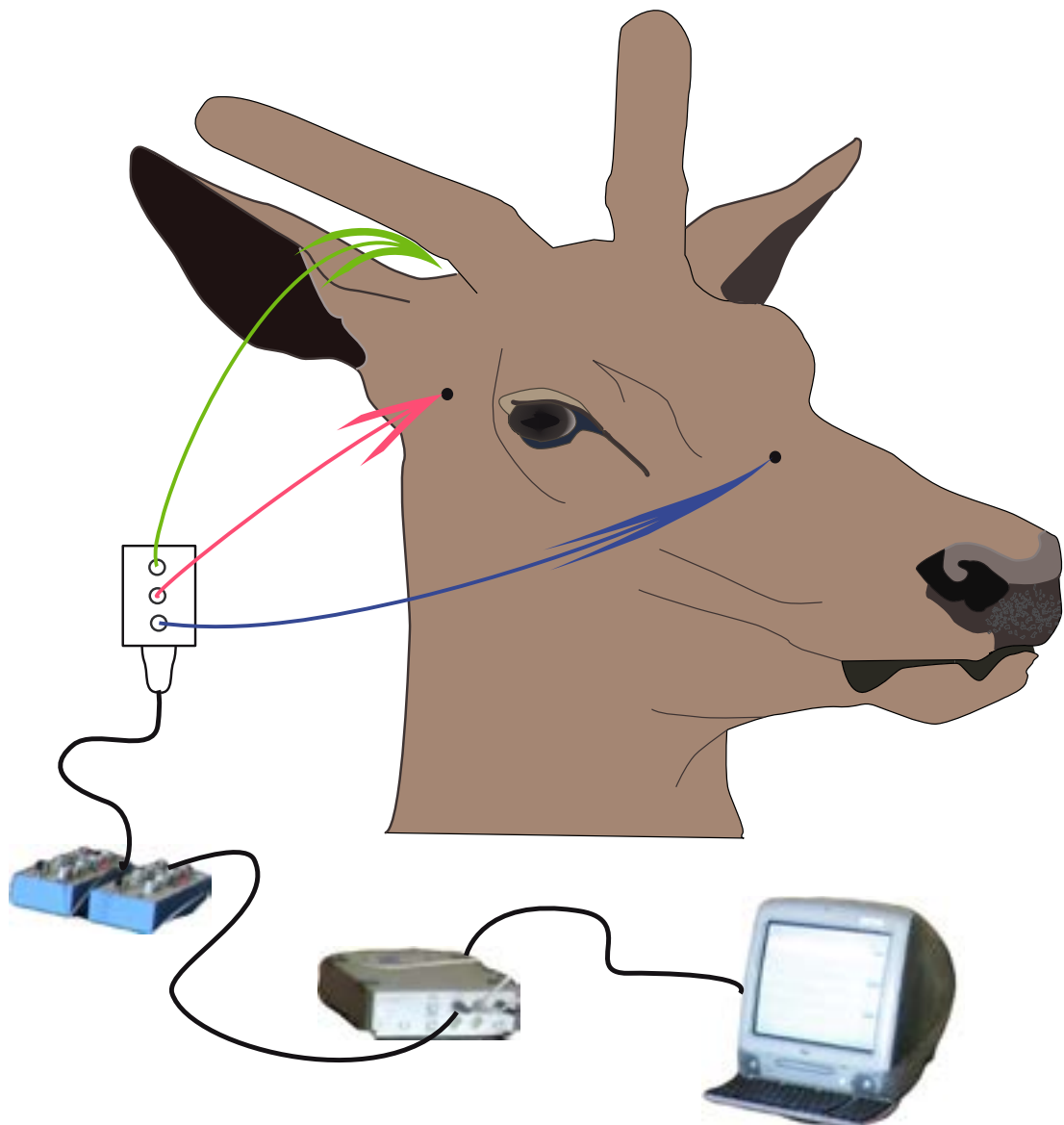


Figure 3.1.4 Diagram showing the positions for placement of the electrodes and connection to the computer recording system for recording electroencephalogram waveforms in anaesthetised yearling red deer during velvet antler removal.

trode was placed subcutaneously over the mastoid process and a reference (active) electrode was placed subcutaneously in the midline over the rostral border of the frontal sinus. A single common (earth) electrode was placed in the subcutaneous tissue immediately caudal to the occipital process in order to isolate the signal from electrical activity generated from the body. This distribution is similar to that described by Johnson et al (2005) in deer, modified from that used in the horse by Johnson and Taylor (1998). Electrodes were used not more than three times and were discarded if poor recordings were observed.

Signals from each channel were fed via a custom built isolating box to individual amplifiers and processed on a Powerlab 4/20 data acquisition system (AD Instruments Ltd.). Raw EEG was recorded continuously using Chart 05 at sampling rate of 1000Hz with a pass band of 0.5 - 400Hz and stored on a Laptop PC (Toshiba). A minimum of five minutes of baseline was recorded prior to cutting of the antler and a minimum of five minutes was recorded after cutting.

Raw EEG was inspected and any artefacts were excluded from further analysis. Fast Fourier transformation (Welch, 1967) was performed using specialised software (FFT program, Craig Johnson) to convert the raw EEG into the frequency domain. Frequency band amplitudes were determined in 1 second epochs from 1000 samples per block from the original sampling. The median frequency, 95% spectral edge frequency and total power of the spectrum in each second was calculated and these data were analysed further for comparison of animals and treatments.

Data analysis

Baseline calculations

For each channel in each animal, baseline means of total power (TP), median frequency (F50) and spectral edge frequency (F95) were calculated over the three minutes immediately prior to antler cutting by determining the mean and variance of the values recorded during this time. This baseline mean was then corrected to 100% so that animals could be compared directly, removing between animal variation in baseline EEG amplitude. Changes in EEG in response to treatment for an individual animal were calculated as % of baseline. The data were analysed unblinded.

15 second means

For statistical comparison of different time periods before and after antler cutting, means were calculated for each 15 second period during the two minutes prior to and five minutes after antler cutting.

ANOVA was performed using Minitab 14 software to compare each 15 second interval with baseline recordings and to compare treatment groups at each 15 second interval. T-tests were performed to determine probability of equal means, with differences considered significant when $p < 0.01$ to allow for repeated comparisons.

Summary of analyses

Pre-cut

Differences from mean baseline within and between groups.

Post-cut

1. Within treatment comparisons

Differences from baseline at each 15 second interval for each of:

- Control
- Lignocaine
- NaturO

2. Between treatment comparisons.

Differences between groups during each 15 second interval for each of:

- Control vs Lignocaine
- Control vs NaturO
- Lignocaine vs NaturO

TP, F50 and F95 were analysed independently

Results

Two anaesthetised animals were unable to be used for recording due to anaesthetic complications. Faulty recordings were produced on two channels in one animal and in one channel in a further three animals. In total, there were 32 (Lig), 33(NaturO) and 32 (Con) sets (channels) of data able to be analysed.

Anaesthesia

Individual animal anaesthetic records are included in Appendix 3.1.1.

Induction

Induction of general anaesthesia was successfully achieved in all animals. One animal went into respiratory and cardiac arrest immediately after induction. Transient apnoea occurred in the majority of stags but this was not recorded due to the difficulty in monitoring respiration during intubation with the stag restrained in the crush (Figure 3.1.1). The dose of induction drugs used was 8.06 ± 1.99 mg/kg of propofol and 5.94 ± 0.54 mg/kg of ketamine. There was greater variation in the propofol dose because propofol was used alone (without ketamine) for top-ups when required.

Intubation

Intubation was achieved in all animals. The time from induction to intubation ranged from less than one minute to 9 minutes. The average time from induction to intubation was 2 minutes. 46 stags were intubated with a size 16 mm ID endotracheal tube and 5 were intubated with a 14 mm

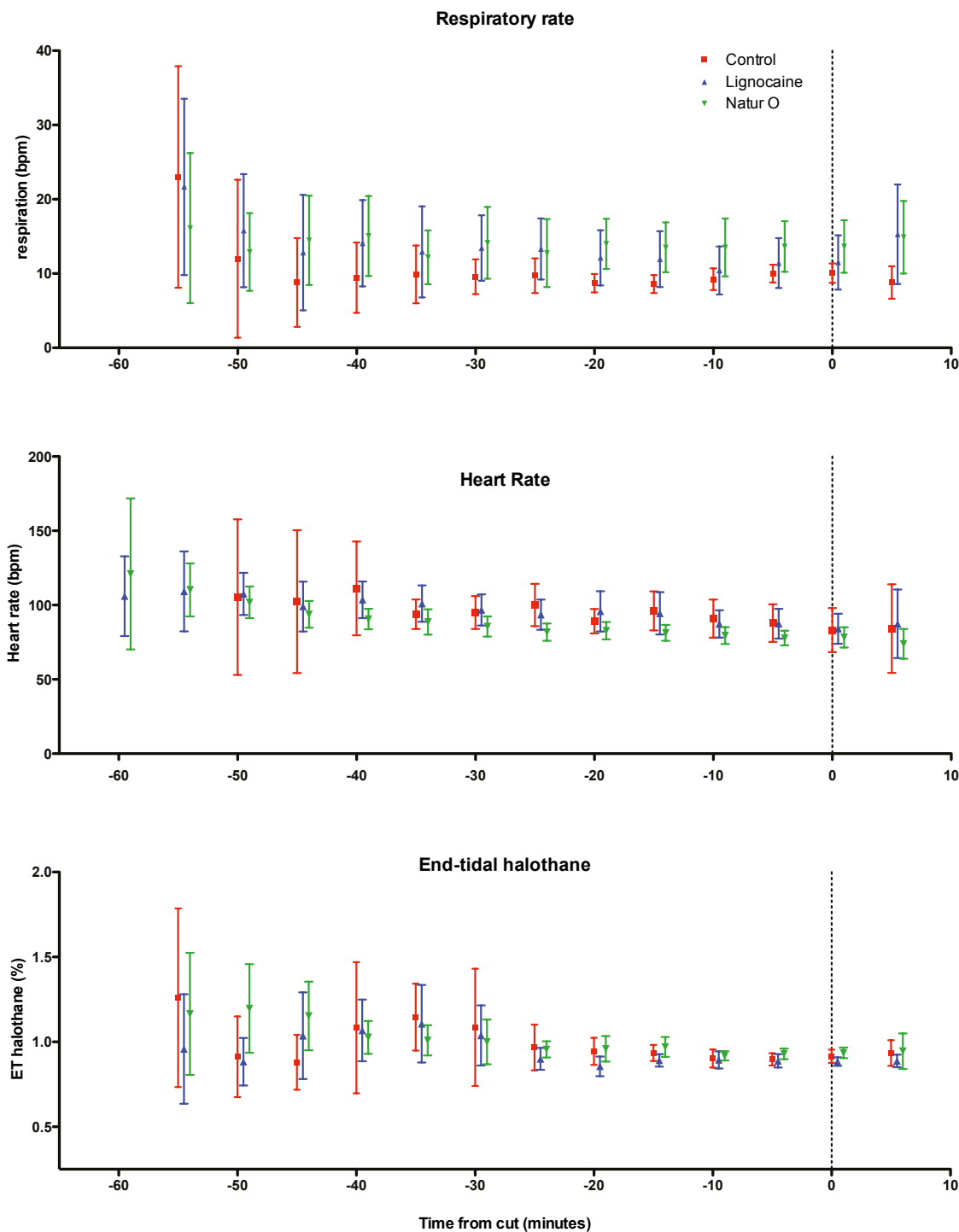


Figure 3.1.5 Mean ($\pm$ 95% CI) Respiratory rate, heart rate and end tidal halothane concentration recorded at five-minute intervals in deer under general anaesthesia for recording of electroencephalogram during velvet antler removal after treatment with either lignocaine(blue), a NaturO™ ring (green) or no treatment (blue). Observation times for each animals have been standardised to the time of cutting (time 0) rather than from induction of anaesthesia.

ID endotracheal tube. Intubation was assisted by having someone grasp the larynx from outside and hold it steady as it was highly mobile and moved away when the tube was pushed against it.

Maintenance

Some animals required several minutes of IPPV as otherwise apnoea, followed by decreased plane of anaesthesia and rapid, shallow breathing was commonly observed. End tidal halothane concentration was stabilised at $0.91 \pm 0.08\%$ of expired gas by 30 minutes after induction and remained at this level for all EEG recordings.

Heart rate and respiratory rate and end-tidal halothane concentration recorded during anaesthesia is shown in Figure 3.1.5. There were no differences between any of the groups in ET halothane concentration or respiratory rate throughout anaesthesia. Control deer had a lower respiratory rate than *NaturO* deer at times -20 and -15 only. There were no differences in respiratory rates between any groups at any other times.

Electroencephalogram

Raw Chart electrical difference waveforms were converted to frequency-amplitude bands using Fast-Fourier transformation. From the frequency spectrum data, the median frequency, 95% spectral edge (F95) and total power (TP) only are shown here. Raw EEG chart files and one-second epoch Fast-Fourier transformed data (frequency range 1-30 Hz) are appended (Appendices 3.1.2 and 3.1.3 respectively)

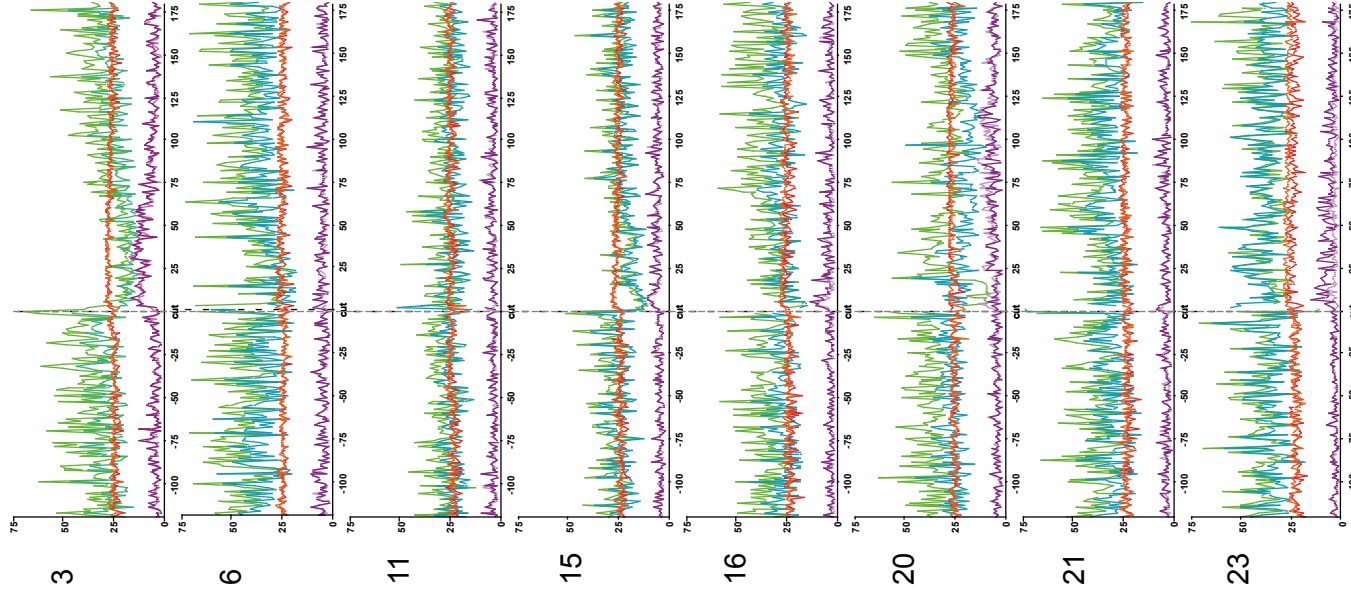
Individual animal time series plots for TP, F50 and F95 with both channels on the same graph are shown in Figure 3.1.6 and the same data converted to percentage of pre-cut baseline are shown in Figure 3.1.7. These graphs show that faulty recordings were made in deer 7(channel 2), deer 24(channel 2), deer 32 (both channels) and deer 41 (channel 2). Descriptions of the visual assessment of individual profiles are given in Table 3.1.1

Visual assessment of individual profiles

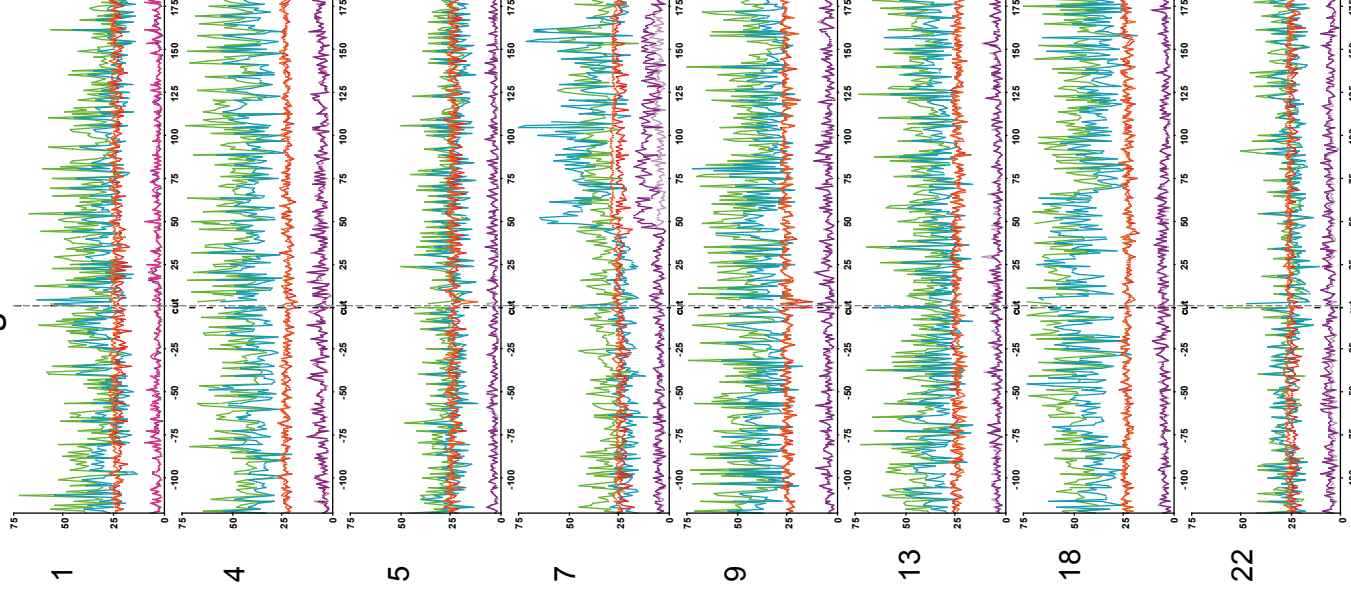
Figure 3.1.6 (pages 172–173) Individual animal time series plots showing electroencephalogram recordings of raw EEG recordings for left and right channels (on the same graph) before and after cutting the antler following treatment with either lignocaine, a NaturO™ ring or no analgesia. One second averages shown.

Figure 3.1.7 (pages 174–175) Individual animal time series plots showing electroencephalogram recordings for TP (green), F50 (purple) and F95 (red) for left (dashed line) and right (solid line) cerebral hemispheres on the same graph before and after cutting the antler following treatment with lignocaine, a NaturO™ ring or no analgesia. Results are as a percentage of pre-cut baseline and averaged over 15 second epochs.

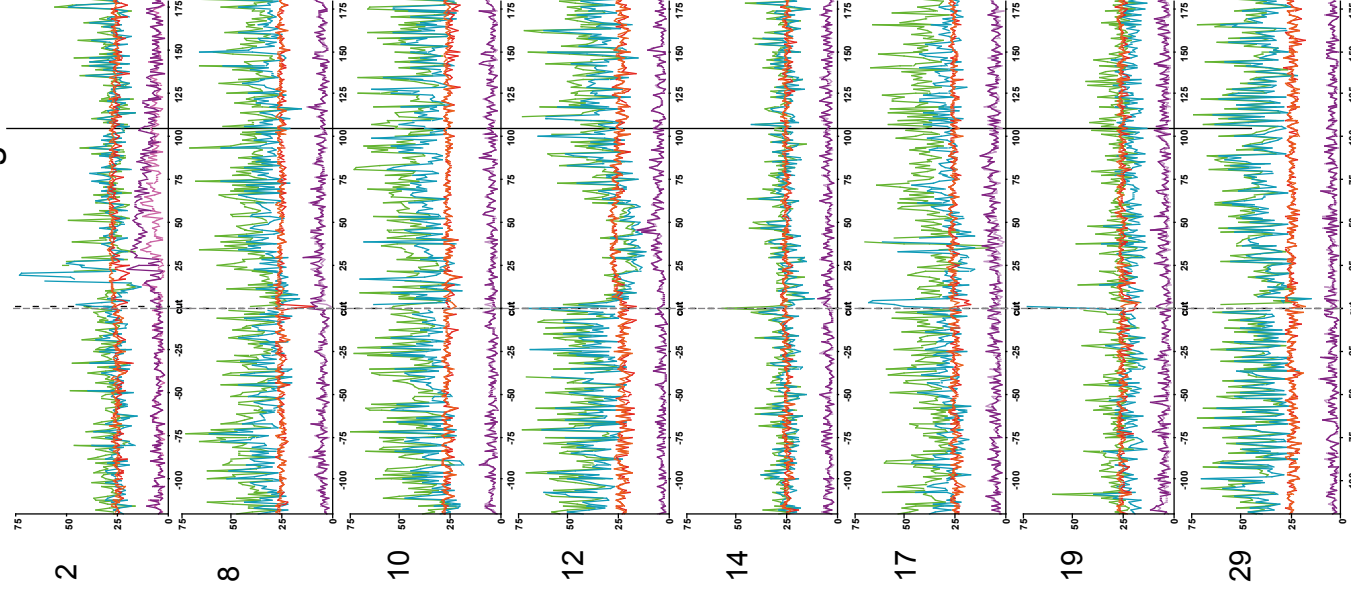
Control

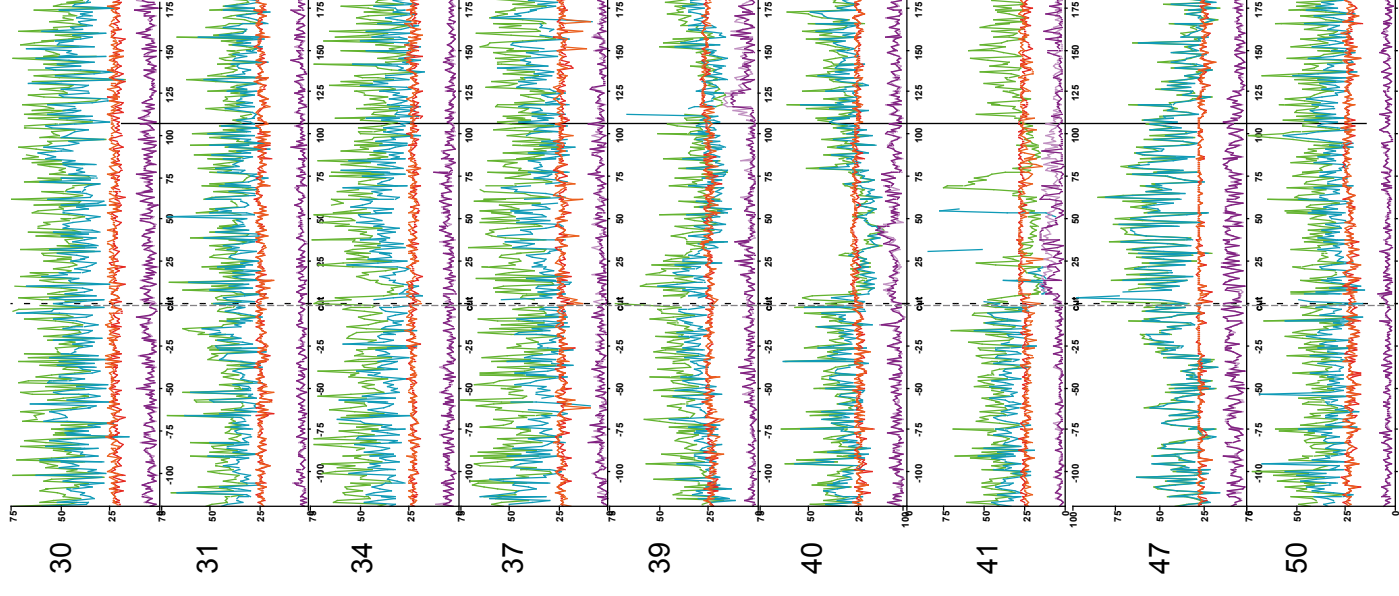
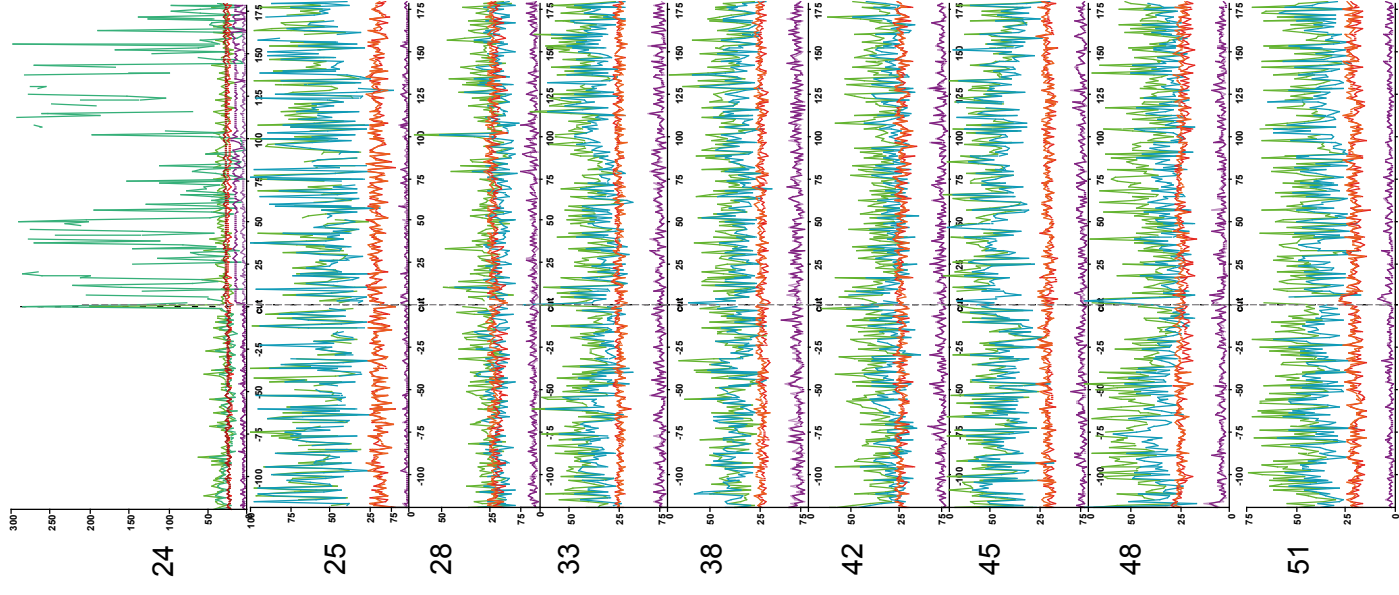
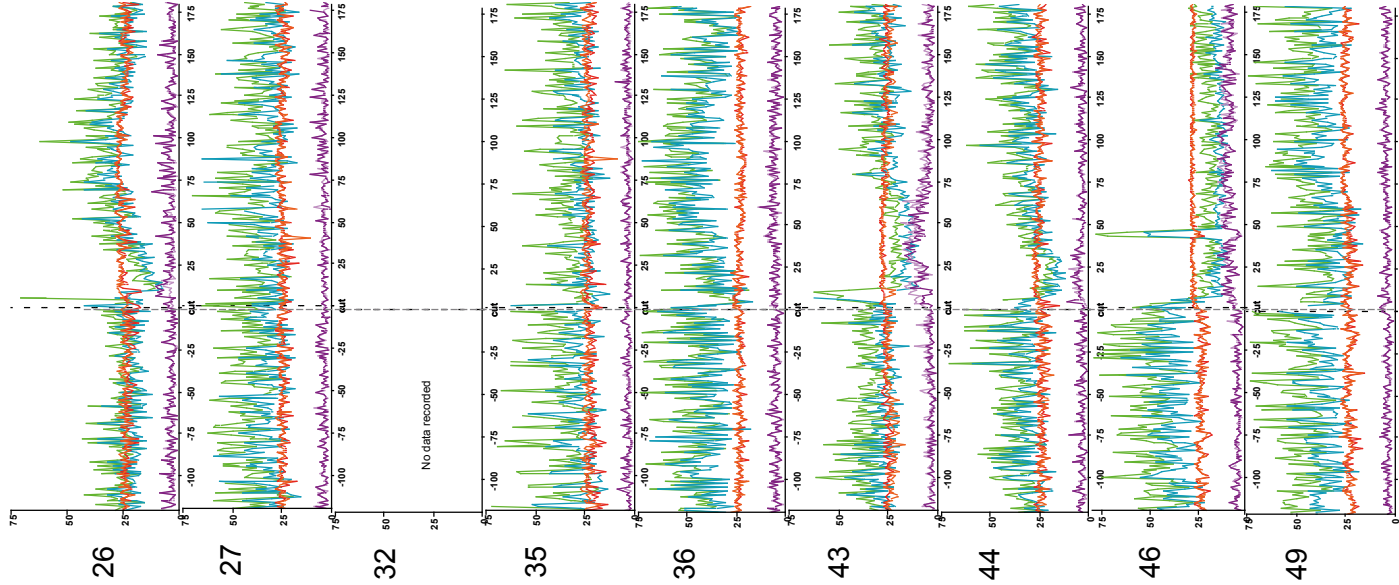


Lignocaine

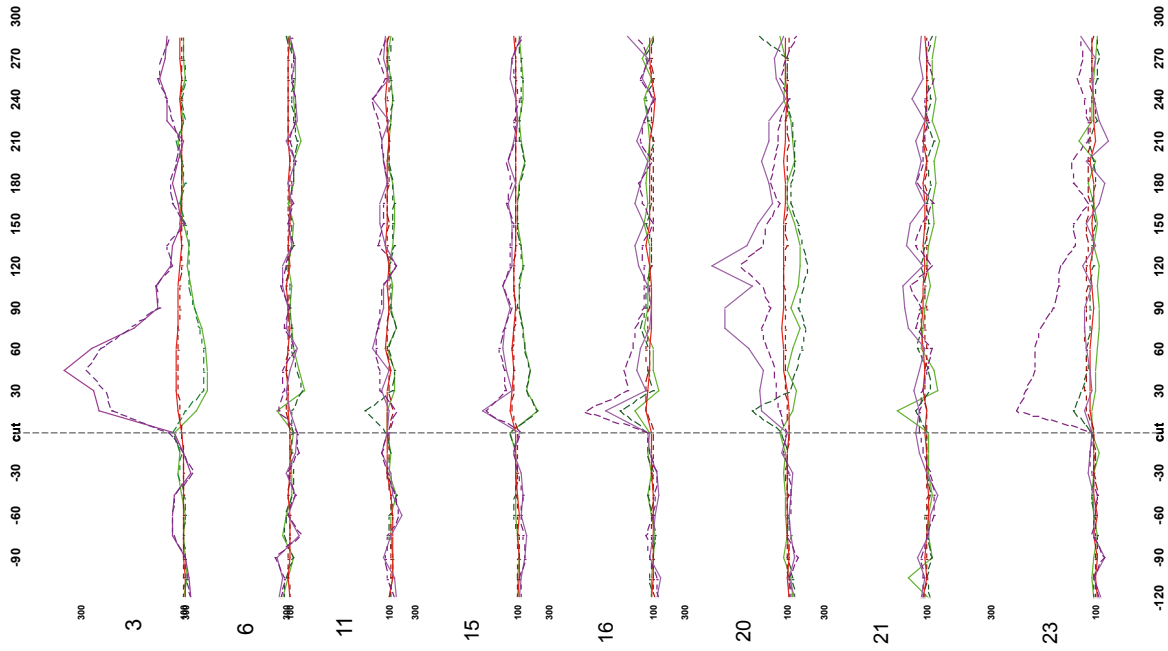


NaturO ring

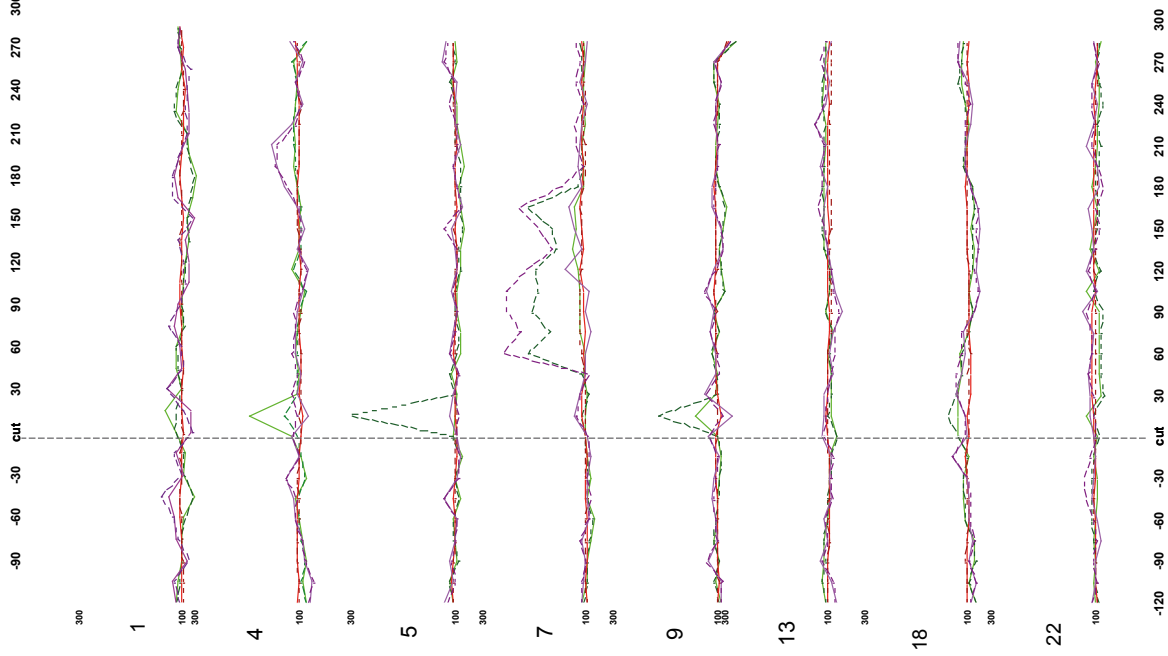




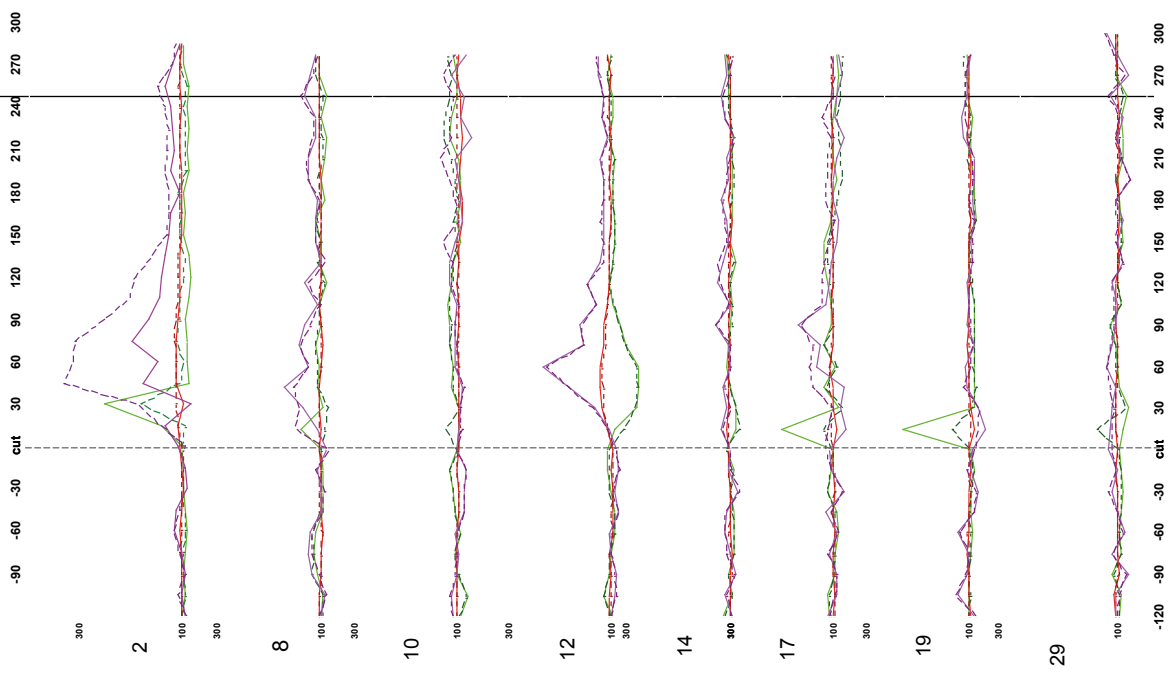
Control



Lignocaine



NaturO ring



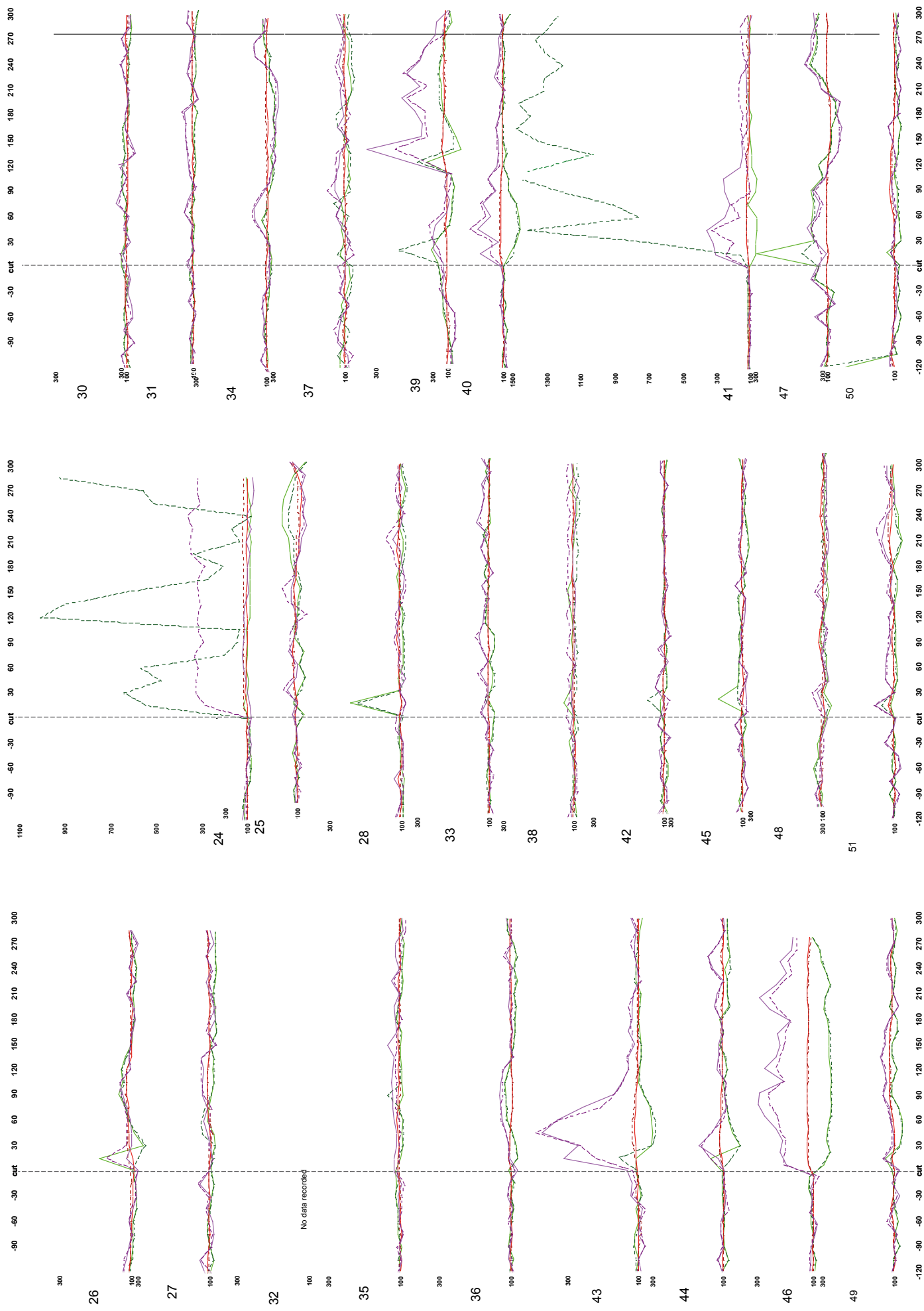


Table 3.1.1 Summary of visual inspection of individual EEG profiles of deer during antler removal following lignocaine (Lig), NaturO™ ring (NO) or no analgesia (Con) to determine whether they were suitable for further analysis.

Obs	ID	Treat	Ch	Check		Obs	ID	Treat	Ch	Check	
1	530	Lig	1	OK		26	210	Con	1	OK	
			2	OK					2	OK	
2	513	NO	1	OK	Movement at cut	27	262	Con	1	OK	
			2	OK	Movement at cut				2	OK	
3	536	Con	1	OK		28	189	Lig	1	OK	
			2	OK					2	OK	
4	510	Lig	1	OK	Large spike at cut	29	218	NO	1	OK	
			2	OK	Large spike at cut				2	OK	
5	588	Lig	1	OK	Large spike at cut	30	539	NO	1	OK	
			2	OK	Large spike at cut				2	OK	
6	507	Con	1	OK		31	569	NO	1	OK	
			2	OK					2	OK	
7	581	Lig	1	OK		32	553	Con	1	Bad	No Data - Wrong sample settings
			2	Bad	(1)				2	Bad	
8	558	NO	1	OK		33	506	Lig	1	OK	
			2	OK					2	OK	
9	262	Lig	1	OK	last 20 secs missing	34	548	NO	1	OK	
			2	OK					2	OK	
10	266	NO	1	OK		35	547	Con	1	OK	
			2	OK					2	OK	
11	189	Con	1	OK		36	582	Con	1	OK	
			2	OK					2	OK	
12	218	NO	1	OK		37	585	NO	1	OK	Ch1 and 2 vary more than usual
			2	OK					2	OK	
13	509	Lig	1	OK		38	584	Lig	1	OK	Baseline drift down (3)
			2	OK					2	OK	
14	594	NO	1	OK		39	210	NO	1	OK	
			2	OK					2	OK	
15	544	Con	1	OK		40	272	NO	1	OK	
			2	OK					2	OK	
16	536	Con	1	OK		41	228	NO	1	OK	(4)
			2	OK					2	Bad	
17	513	NO	1	OK	tail off right at end	42	205	Lig	1	OK	
			2	OK	tail off right at end				2	OK	
18	510	Lig	1	OK		43	183	Con	1	OK	
			2	OK					2	OK	
19	588	NO	1	OK		44	267	Con	1	OK	
			2	OK					2	OK	
20	558	Con	1	OK		45	200	Lig	1	OK	
			2	OK					2	OK	
21	507	Con	1	OK		46	261	Con	1	OK	
			2	OK					2	OK	
22	581	Lig	1	OK		47	174	NO	1	OK	large move spike
			2	OK					2	OK	
23	509	Con	1	OK		48	157	Lig	1	OK	
			2	OK					2	OK	
24	544	Lig	1	OK		49	274	Con	1	OK	
			2	Bad	(2)				2	OK	
25	597	Lig	1	OK		50	182	NO	1	OK	Large spike in baseline 2'30" before cut
			2	OK					2	OK	
						51	164	Lig	1	OK	
									2	OK	

Notes from Table 3.1.1 (previous page)

- (1) Artefact increase in TP and high freq not in channel 1
- (2) Erratic and bizarre recordings probably electrode interference
- (3) Increased activity late after cut consistent both channels
- (4) Large and bizarre readings

In the control group 10 out of 16 animals showed changes indicative of a cerebral response to painful somatosensory stimulus. This is characterised by an increase in median and/or spectral edge frequency and a decrease in total power. None of the animals in the Lignocaine group showed changes characteristic of somatic noxious stimulus and five animals in the *NaturO*TM ring group showed characteristic changes after cutting the antler.

Treatment group results

Figure 3.1.8 shows a time series plots of mean TP, F50 and F95 as a percentage of baseline for each treatment group from two minutes prior to cutting to five minutes after cutting.

Table 3.1.2 gives values for the mean, standard deviation and standard error of the mean for TP, F50 and F95 (as a percentage of the baseline) for each treatment group in 15 second intervals from two minutes prior to cutting to five minutes after cutting. Table 3.1.3 give p values for t-tests for equal means between groups and within a group compared to pre-cut baseline. Where means differed significantly, the cells are highlighted in yellow. The following is a descriptive summary of the data in these tables and on the graph in Figure 3.1.8.

Pre-cut

Baseline recordings were stable during the two minute pre-cut recordings for all parameters. Significant fluctuation in baseline from the mean are highlighted in yellow in Table 3.1.3

Total Power (TP)

During the two minutes immediately prior to cutting the antler, significant difference in total power were calculated as follows. At -115 seconds Con was 2.6% less than baseline ($p < 0.05$) and 4.4% less than *NaturO* ($p < 0.01$), at -30 seconds LA was 5.2% less than baseline ($p < 0.001$), 6.8% less than *NaturO* ($p < 0.01$) and 5.8% less than Con ($p < 0.01$) and at -15 seconds Con was 5.3% above baseline ($p < 0.01$).

Post-cut

Within treatment comparisons (TP)

After cutting, there were significant changes in total power, particularly in the control group.

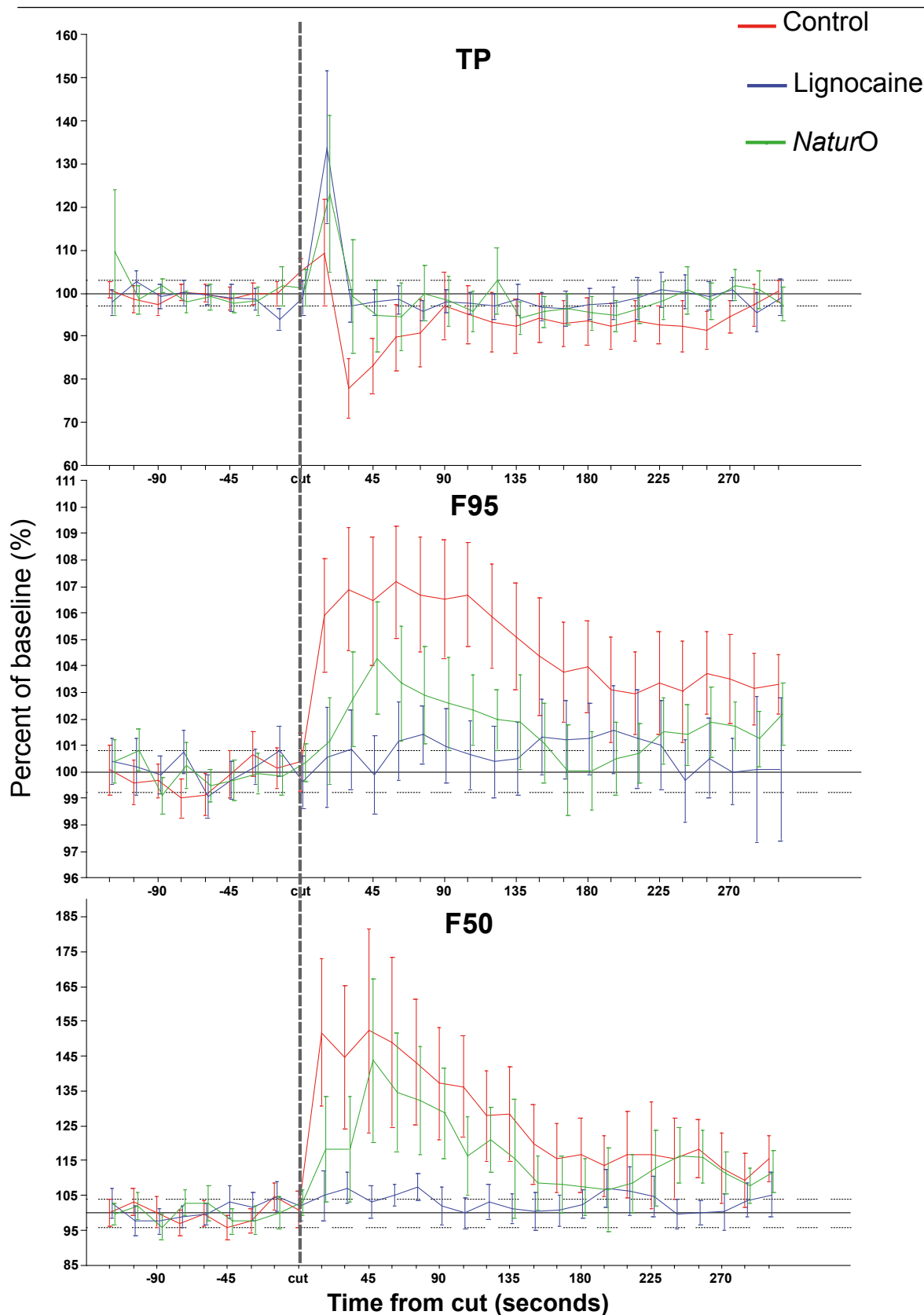


Figure 3.1.8 Mean and 95% confidence interval of the mean) Total Power, F95 and F50 recorded from yearling stags under general anaesthesia before and after cutting antler after either no analgesia (Control), Lignocaine (LA) or an NaturO for analgesia. Data is plotted at percentage of baseline value for each deer. 100% = baseline and the dashed lines either side of 100% represent the 95% confidence interval of the baseline recordings.

Control: Post-cut differences from baseline

In the Con group, during the first 15 seconds, TP increased above baseline, but was not statistically significant. Between 15 and 30 seconds after cutting, the total power had dropped sharply to a negative peak of 22% below baseline ($p < 0.000001$). This was followed by a steady return, being 17% ($p < 0.00001$), 10% ($p < 0.01$) and 9% ($p < 0.05$) below baseline at 30, 45, and 60 seconds respectively. Mean TP remained below baseline for the entire post cut period. This difference was not significant between 75 and 105 seconds over which period the difference was between 3 and 7%. From 2 minutes until 4.5 minutes, there was a significant and sustained difference from baseline, reaching a maximum of 8.6% at 4 minutes. During the final one minute of recording, TP again rose steadily back to baseline level which was reached at 5 minutes.

LA: Post-cut differences from baseline

For the LA group, the only change in total power was a large rise of 33% above baseline during antler cutting. After this time TP was not significantly different from baseline at any point.

NaturO: Post-cut differences from baseline

The NaturO group showed a pattern of change similar to LA during cutting and similar to Con group thereafter. These changes were neither as large or significant in either direction. Mean TP was 23% above baseline during cutting ($p < 0.05$) and then dropped below baseline and remained there for all except four of the 15 second intervals. This decrease was significant ($p < 0.05$) at 120, 135, 165 and 180 seconds and did not demonstrate any clear pattern over time.

Between treatment comparisons (TP)*Con vs LA: Post-cut differences between groups*

TP in the Con group was significantly less than LA by 25% ($p < 0.05$), 19% ($p < 0.000001$), and 16% ($p < 0.00001$) at 0, 15, and 30 seconds respectively and by 8% ($p < 0.01$), 8% ($p < 0.05$), 9% ($p < 0.01$), and 6% ($p < 0.01$) at 210, 225, 240 and 255 seconds respectively. The latter four time periods corresponded with a decrease in TP for the Con group.

Con vs NaturO: Post-cut differences between groups

Mean TP for the Con group was less than that of the NaturO group for the entire recording period. The difference was significant at 15 and 30 seconds, when Con was 21% ($p < 0.01$) and 12% ($p < 0.05$) less than NaturO. Not significant between 45 and 210 seconds, and significantly less than NaturO by 9% ($p < 0.05$), 7% ($p < 0.05$) and 7% ($p < 0.01$) at 225, 240 and 255 seconds respectively.

LA vs NaturO: Post-cut differences between groups

TP in the LA group was not significantly different from the *NaturO* group at any time.

Median Frequency (F50)

Precut

There were no significant differences between groups.

Control: Post-cut differences from baseline

F50 in the control group was significantly greater than baseline for the entire 5 minutes after cutting. An increase of 50% was seen in the first 15 seconds ($p<0.0001$) and this reached a peak of 52% above baseline between 30 and 45 seconds ($p<0.001$). This was followed by a gradual decline towards baseline. By 120 seconds, F50 was 28% above baseline ($p<0.001$) and by four minutes this had decreased to 17% above baseline ($p<0.01$). By the end of recording median frequency was still at least 9% above baseline ($p<0.05$).

LA: Post-cut differences from baseline

F50 was significantly greater than baseline in the LA group at four irregularly distributed time periods after cutting. F50 was 7.3% ($p<0.01$), 10% ($p<0.05$) and 6.7% ($p<0.05$) and 6.5% ($p<0.01$) greater than baseline at 15, 60, 180, and 270 seconds after cutting respectively. The shape of the F50 curve after cutting was different from that of Con animals. There was no clear rise immediately after cutting followed by a gradual decline as was seen in the Con group.

NaturO: Post-cut differences from baseline

Changes in the *NaturO* group were intermediate of those seen in the LA and Con groups. Median frequency increased by approximately 15% ($p<0.05$) during the first 30 seconds after cutting and then peaked at 38% ($p<0.001$) above baseline between 30 and 45 seconds. There was a gradual decline to 21% ($p<0.001$) above baseline by 2 minutes. After this time there was a one and a half minute period during which median frequency was slightly, but not significantly above baseline, followed by a smaller rise to around 16% ($p<0.001$) above baseline at four minutes. This remained significantly elevated until the end of recording ($p<0.001$)

Between treatment comparisons

Con vs LA : Post-cut differences between groups

F50 in the Con group was greater than that of the LA group by 46% ($p<0.001$), 37% ($p<0.001$), 50% ($p<0.01$), 39% ($p<0.01$), 32% ($p<0.01$), 31% ($p<0.001$), 32% ($p<0.001$), 22% ($p<0.01$), 25% ($p<0.001$) 17% ($p<0.05$) at 0, 15, 30, 45, 60, 75, 90, 105, 120, and 135 seconds after cutting respectively and 16% ($p<0.01$), 18% ($p<0.001$), 13% ($p<0.05$) and 10% ($p<0.05$) greater than the

LA group at 225, 240, 255, and 285 seconds respectively.

Con vs NaturO: Post-cut differences between groups

Differences between Con and *NaturO* were not as large as those demonstrated between Con and LA. Con was 36% ($p < 0.01$) and 29% ($p < 0.05$) greater than *NaturO* by 15 and 30 seconds respectively. After this time, *NaturO* remained consistently lower than Con for all time periods. These differences were not statistically significant ($p > 0.05$).

NaturO vs LA: Post-cut differences between groups

F50 in the *NaturO* group was higher than that of the LA group for all except two time intervals after antler cutting. The difference was not statistically significant until 30 seconds, when *NaturO* F50 was 36% greater than that of LA ($p < 0.01$). *NaturO* remained significantly greater than LA by 24% ($p < 0.05$), 19% ($p < 0.05$), 24% ($p < 0.01$) and 15% ($p < 0.05$) at 45, 60, 75 and 105 seconds, respectively. Between 2 minutes and 3 and a half minutes after cutting, the difference between LA and *NaturO* was not significant. A rise in F50 in the *NaturO* group between 3 and a half and 4 and a half minutes coincided with a significant increase above LA of 18% ($p < 0.001$), 16% ($p < 0.001$) and 11% ($p < 0.01$) at 225, 240 and 255 seconds, respectively.

95% spectral edge frequency (F95)

There were smaller absolute changes in F95 than F50. Where differences were seen they were often highly significant ($p < 0.001$), especially within the Con group.

Pre-Cut

There was less variation in F95 during baseline recording compared with F50 and TP. Significant differences seen in the two minutes prior to cutting were at -105 seconds when the *NaturO* group was 0.9% below baseline ($p < 0.01$) and at -90 seconds when Con was 0.9% below baseline ($p < 0.01$) and Con was 1.6% less than LA ($p < 0.01$).

Post-cut (F95)

Within treatment comparisons

Control: Post-cut differences from baseline

F95 increased significantly after cutting compared to the two minute baseline for the entire 5 minutes of recording ($p < 0.001$). F95 increased rapidly within the first 15 seconds to 6.5% above baseline ($p < 0.00001$) and continued to rise to a peak of 7.4% above baseline between 45 and 60 seconds after cutting ($p < 0.0000001$). F95 remained at about this level until 90 seconds after cutting, then declined steadily to around 4% above baseline ($p < 0.001$) over the next two minutes.

Table 3.1.2 Statistical summary of EEG changes in TP, F50 and F95 as a percent of baseline, averaged over 15 second epochs before and after cutting the velvet antler of yearling stags provided with either lignocaine, a NaturO™ ring or no analgesia prior to cutting.

The table on this page shows average results for animals in the control group. The results for Lignocaine and NaturO™ ring groups are shown on the following two pages respectively.

Control									
Time (sec)	Mean	TP SD	SEM	Mean	F50 SD	SEM	Mean	F95 SD	SEM
-120	98.6	9.1	1.6	103	10	1.8	99.6	2.4	0.42
-105	97.4	6.8	1.2	100	12	2.2	99.9	2	0.36
-90	100.1	5.4	0.96	97	10	1.7	99.1	1.9	0.34
-75	100	5.3	0.9	100	10	1.8	99.5	2.2	0.39
-60	98.7	7.6	1.3	96	10	1.7	100.2	2.2	0.4
-45	99.9	7.1	1.2	98	10	1.7	100.7	2.2	0.39
-30	99.8	7.7	1.4	105	11	1.9	100.4	2.2	0.39
-15	105.2	7.5	1.3	101	14	2.6	100.6	2.7	0.48
		Cut			Cut			Cut	
15	109.4	34.2	6	152	59	10.4	106.6	5.9	1.05
30	77.9	18.8	3.3	145	57	10.1	107	6.6	1.16
45	83.2	18	3.2	152	81	14.4	106.6	6.3	1.12
60	89.7	21	3.7	149	68	12	107.4	6	1.05
75	90.8	21.7	3.8	143	50	8.9	107	5.9	1.04
90	97	21.5	3.8	137	45	7.9	106.8	6.4	1.13
105	95.1	18.8	3.3	136	41	7.2	106.8	5.4	0.96
120	93.2	19.2	3.4	128	36	6.4	106	5.4	0.95
135	92.3	17.1	3	128.3	37.2	6.6	105.3	5.6	0.98
150	94.2	15.8	2.8	119.7	31.8	5.6	104.6	5.9	1.05
150	93	14.9	2.6	115.6	27.4	4.8	103.8	5.3	0.94
165	93.5	15.3	2.7	116.6	29.5	5.2	104.1	4.9	0.86
180	92.4	14.9	2.6	113.5	24.1	4.3	103.3	5.1	0.9
195	93.6	13.3	2.4	116.8	34.2	6.1	103.3	4.5	0.79
210	92.7	11.8	2.1	116.5	42.5	7.5	103.5	5.3	0.93
225	92.3	16.5	2.9	115.5	32.3	5.7	103.1	5.3	0.93
240	91.4	12.2	2.1	118.4	23.3	4.1	104.1	4.1	0.72
255	94.7	10.4	1.8	112.9	28	4.9	103.8	4.5	0.79
270	97.2	13.5	2.4	109.3	21.4	3.8	103.2	3.9	0.69
285	100.5	7.3	1.3	115.4	18.3	3.2	103.6	3	0.53

Table 3.1.2 continued: The table on this page shows average results for animals in the lignocaine group. The results for Control and NaturO™ ring groups are shown on the previous and following pages respectively.

Lignocaine									
Time (sec)	Mean	TP SD	SEM	Mean	F50 SD	SEM	Mean	F95 SD	SEM
-120	102.6	7.1	1.2	98	12	2.1	99.9	2.8	0.5
-105	98.9	7.7	1.4	98	10	1.8	100.1	2.1	0.37
-90	99.7	8.5	1.5	100	9	1.5	100.6	2.3	0.4
-75	99.2	6.6	1.2	100	8	1.4	99.3	2	0.36
-60	98.9	8.5	1.5	103	13	2.2	99.7	1.9	0.34
-45	98.8	6.6	1.2	101	11	2	99.8	1.9	0.33
-30	94.2	7.1	1.3	104	12	2.1	100.8	3.1	0.55
-15	99.4	10.9	1.9	101	11	2	99.4	3	0.54
	Cut			Cut			Cut		
15	133.8	49.5	8.7	105	20	3.5	100.9	5.5	0.98
30	97.4	10.2	1.8	107	12	2.1	100.6	4	0.72
45	98.6	8.1	1.4	102	12	2.1	100	4	0.7
60	102.2	22.1	3.9	110	29	5.1	101.4	4.3	0.77
75	98.5	14.1	2.5	111	24	4.1	102	3.5	0.63
90	101.9	20.2	3.6	106	30	5.3	100.8	4.3	0.76
105	100.3	18.2	3.2	104	30	5.3	101.1	4.5	0.79
120	99.7	19.7	3.5	106.3	24.3	4.3	100.7	4.1	0.73
135	100.1	14.6	2.6	103	16	2.8	100.8	3.9	0.68
150	98.9	15.3	2.7	102.9	22.1	3.9	101.4	4.1	0.73
150	99.6	23.7	4.2	105.2	26	4.6	101.5	4.4	0.78
165	97.8	10.6	1.9	103.8	13.4	2.4	101.4	4	0.71
180	97.7	10.3	1.8	106.7	14.7	2.6	101.7	4.2	0.74
195	98.5	13.1	2.3	106.6	19.1	3.4	101.4	5.3	0.94
210	101.1	11.1	1.9	104.5	16.1	2.8	100.8	4.8	0.85
225	100.5	11.1	2	99.2	11	1.9	99.8	4.9	0.87
240	99.7	9.1	1.6	100.3	10.4	1.8	100.8	3.8	0.67
255	101.1	7.7	1.4	100.2	14.2	2.5	100.2	3.4	0.6
270	97.8	10	1.8	106.5	11.3	2.1	101.6	5.5	1
285	99.3	11.4	2.1	104.9	16.9	3.1	101.9	4.9	0.89

Table 3.1.2 continued: The table on this page shows average results for animals in the *NaturO* group. The results for Control and Lignocaine groups are shown on the previous two pages.

<i>NaturO</i>									
Time (sec)	Mean	TP SD	SEM	Mean	F50 SD	SEM	Mean	F95 SD	SEM
-120	98.6	9.4	1.6	102	11	2	100.7	2.1	0.37
-105	101.8	4.5	0.78	96	11	1.9	99.1	2.1	0.36
-90	97.9	6.9	1.2	103	11	1.8	100	2.2	0.38
-75	99.1	8.4	1.5	103	14	2.4	99.8	1.8	0.31
-60	97.7	6.1	1.1	98	11	1.9	99.7	2.2	0.38
-45	98.2	9.1	1.6	98	11	2	99.9	2.1	0.37
-30	101.6	12.9	2.2	100	14	2.4	100.1	2.1	0.37
-15	101.2	11.8	2.1	103	11	1.9	100.6	2.1	0.37
	Cut			Cut			Cut		
15	123	51.5	9	115	39	6.7	101.2	4.8	0.83
30	99.2	37.3	6.5	116	42	7.3	102.6	5.7	0.99
45	94.7	23.2	4	139	61	10.6	104.2	5.9	1.03
60	94.5	21.8	3.8	134	49	8.6	102.9	6.4	1.11
75	100	18	3.1	130	43	7.5	102.9	5.2	0.9
90	98.2	16.3	2.8	130	37	6.5	102.8	4.9	0.86
105	95.8	13	2.3	117	32	5	102.4	3.9	0.68
120	102.9	21.5	3.7	121.3	26.9	4.7	101.7	3.3	0.56
135	94.3	10.7	1.9	115.5	49.4	8.6	101.9	4.9	0.85
150	95.6	10.1	1.8	107.9	22.1	3.9	100.7	4.2	0.73
150	96.3	10	1.7	106.9	22.9	4	100	4.6	0.8
165	95.3	10.7	1.9	106.7	23.4	4.1	99.9	4	0.7
180	94.7	10.5	1.8	105.1	34.2	6	100.1	3	0.6
195	96.3	9.5	1.7	107	22.3	3.9	100.5	3.1	0.54
210	98.3	12.2	2.1	111.7	30.9	5.4	101.6	3.4	0.59
225	100.7	15.4	2.7	116.7	23.1	4	101.5	3.3	0.58
240	98.2	11.5	2	115.5	21.6	3.8	101.7	3.8	0.66
255	101.8	10.4	1.8	110.6	16.9	2.9	101.5	2.3	0.4
270	100.8	12	2.1	106.4	12.6	2.2	101.2	3.2	0.55
285	97.5	10.6	1.9	112.1	17.1	3.1	102	3.4	0.62

Table 3.1.3 *p*-values generated from *t*-tests for comparison of equal means of EEG data for Total Power, Median Frequency and 95% spectral edge frequency data recorded during cutting the antlers of anaesthetised stags following analgesia treatment with either lignocaine, a NaturO™ ring or no analgesia.

Time is shown in the left hand column and is referenced from the time of antler cutting (Cut). Precut recording are termed “baseline”. Comparisons between groups were made at the same time period except when the group was compared to itself. In this case, the comparison was made between the specified time period and the average baseline recording for that group. Comparisons within and between treatment groups are shown for TP, F50 and F95. Significant differences are highlighted in yellow. Differences were considered significant when $p < 0.01$. The table on this page shows the results for the Control group. Results for Lignocaine and NaturO groups are shown on the following two pages respectively

Control									
Time	Control vs Control			Control vs Lignocaine			Control vs NaturO		
	TP	F50	F95	TP	F50	F95	TP	F50	F95
	Baseline			Baseline			Baseline		
-120	0.3	0.1	0.2	0.05	0.06	0.7	0.1	0.7	0.07
-105	0.02	0.9	0.4	0.4	0.4	0.7	0.003	0.2	0.1
-90	0.7	0.1	0.004	0.8	0.2	0.005	0.2	0.02	0.07
-75	0.6	0.9	0.1	0.6	0.9	0.6	0.6	0.3	0.6
-60	0.2	0.03	0.9	0.9	0.01	0.4	0.6	0.5	0.4
-45	0.7	0.2	0.2	0.5	0.2	0.09	0.4	0.8	0.2
-30	0.6	0.02	0.6	0.003	0.9	0.6	0.5	0.1	0.6
-15	0.001	0.7	0.5	0.01	0.9	0.1	0.1	0.5	0.9
	CUT			CUT			CUT		
15	0.2	0.00002	0.000001	0.02	0.0001	0.0001	0.2	0.004	0.0002
30	0	0.0001	0.000002	0.000005	0.001	0.00002	0.005	0.03	0.005
45	0.000006	0.001	0.000003	0.00006	0.002	0.000007	0.03	0.4	0.1
60	0.007	0.0003	0.000000	0.02	0.005	0.00003	0.4	0.3	0.005
75	0.02	0.00003	0.000000	0.1	0.002	0.0001	0.1	0.3	0.005
90	0.4	0.00005	0.000002	0.4	0.002	0.00007	0.8	0.5	0.007
105	0.1	0.00002	0.000000	0.3	0.0007	0.00003	0.9	0.04	0.0004
120	0.04	0.0001	0.000001	0.2	0.007	0.00003	0.06	0.4	0.0003
135	0.01	0.0002	0.00001	0.06	0.001	0.0003	0.6	0.2	0.01
150	0.03	0.001	0.0002	0.2	0.02	0.02	0.7	0.09	0.003
150	0.008	0.003	0.0007	0.2	0.1	0.06	0.3	0.2	0.003
165	0.02	0.003	0.00008	0.2	0	0.02	0.6	0.1	0.0003
180	0.005	0.003	0.002	0.1	0.2	0.2	0.5	0.3	0.004
195	0.007	0.009	0.0006	0.1	0.2	0.1	0.3	0.2	0.006
210	0.0008	0.03	0.001	0.005	0.1	0.03	0.06	0.6	0.09
225	0.008	0.01	0.005	0.02	0.01	0.01	0.04	0.9	0.2
240	0.0002	0.00009	0.000007	0.003	0.0002	0.001	0.03	0.6	0.02
255	0.004	0.01	0.00009	0.006	0.03	0.0006	0.007	0.7	0.01
270	0.2	0.02	0.0002	0.8	0.5	0.2	0.3	0.5	0.03
285	1	0.00004	0	0.6	0.02	0.1	0.2	0.5	0.06

Table 3.1.3 continued. The table on this page shows the results for the Lignocaine group. Results for Control and *NaturO* groups are shown on the previous and following two pages respectively

Lignocaine									
Time	Lignocaine vs Control			Lignocaine vs Lignocaine			Lignocaine vs <i>NaturO</i>		
	TP	F50	F95	TP	F50	F95	TP	F50	F95
	Baseline			Baseline			Baseline		
-120	0.05	0.06	0.7	0.01	0.3	1	0.05	0.1	0.2
-105	0.4	0.4	0.7	0.9	0.3	0.6	0.08	0.5	0.06
-90	0.8	0.2	0.005	0.8	0.9	0.09	0.4	0.1	0.3
-75	0.6	0.9	0.6	0.9	0.7	0.1	1	0.2	0.3
-60	0.9	0.01	0.4	0.9	0.2	0.6	0.5	0.06	0.9
-45	0.5	0.2	0.09	0.8	0.5	0.9	0.8	0.3	0.8
-30	0.003	0.9	0.6	0.0006	0.05	0.1	0.006	0.2	0.3
-15	0.01	0.9	0.1	0.9	0.6	0.4	0.5	0.5	0.07
	CUT			CUT			CUT		
15	0.02	0.0001	0.0001	0.0004	0.1	0.3	0.4	0.2	0.8
30	0.000005	0.001	0.00002	0.3	0.002	0.3	0.8	0.2	0.1
45	0.00006	0.002	0.000007	0.7	0.4	0.9	0.3	0.002	0.001
60	0.02	0.005	0.00003	0.5	0.06	0.06	0.2	0.02	0.3
75	0.1	0.002	0.0001	0.8	0.01	0.002	0.7	0.03	0.4
90	0.4	0.002	0.00007	0.5	0.3	0.2	0.4	0.006	0.1
105	0.3	0.0007	0.00003	0.8		0.1	0.3	0.1	0.2
120	0.2	0.007	0.00003	0.9	0.2	0.3	0.5	0.02	0.3
135	0.06	0.001	0.0003	0.8	0.3	0.2	0.08	0.2	0.3
150	0.2	0.02	0.02	0.9	0.5	0.04	0.3	0.4	0.5
150	0.2	0.1	0.06	0.9	0.3	0.05	0.5	0.8	0.2
165	0.2	0	0.02	0.5	0.1	0.04	0.3	0.5	0.12
180	0.1	0.2	0.2	0.4	0.01	0.02	0.3	0.8	0.09
195	0.1	0.2	0.1	0.8	0.06	0.1	0.4	0.9	0.4
210	0.005	0.1	0.03	0.3	0.1	0.3	0.3	0.2	0.4
225	0.02	0.01	0.01	0.5	0.7	0.9	0.9	0.0003	0.09
240	0.003	0.0002	0.001	0.8	0.9	0.2	0.6	0.0007	0.3
255	0.006	0.03	0.0006	0.2	0.9	0.6	0.8	0.009	0.06
270	0.8	0.5	0.2	0.5	0.005	0.1	0.3	0.98	0.7
285	0.6	0.02	0.1	1	0.1	0.03	0.5	0.1	1

Table 3.1.3 continued The table on this page shows the results for the *NaturO* group. Results for Control and Lignocaine groups are shown on the previous two pages

<i>NaturO</i>									
Time	<i>NaturO</i> vs Control			<i>NaturO</i> vs Lignocaine			<i>NaturO</i> vs <i>NaturO</i>		
	TP	F50	F95	TP	F50	F95	TP	F50	F95
	Baseline			Baseline			Baseline		
-120	0.1	0.7	0.07	0.05	0.1	0.2	0.5	0.4	0.3
-105	0.003	0.2	0.1	0.08	0.5	0.06	0.1	0.06	0.007
-90	0.2	0.02	0.07	0.4	0.1	0.3	0.2	0.1	0.7
-75	0.6	0.3	0.6	1	0.2	0.3	0.9	0.2	0.4
-60	0.6	0.5	0.4	0.5	0.06	0.9	0.1	0.3	0.4
-45	0.4	0.8	0.2	0.8	0.3	0.8	0.5	0.4	0.6
-30	0.5	0.1	0.6	0.006	0.2	0.3	0.4	0.9	0.6
-15	0.1	0.5	0.9	0.5	0.5	0.07	0.4	0.1	0.2
	CUT			CUT			CUT		
15	0.2	0.004	0.0002	0.4	0.2	0.8	0.02	0.04	0.2
30	0.005	0.03	0.005	0.8	0.2	0.1	1	0.04	0.01
45	0.03	0.4	0.1	0.3	0.002	0.001	0.3	0.0009	0.0003
60	0.4	0.3	0.005	0.2	0.02	0.3	0.2	0.0003	0.01
75	0.1	0.3	0.005	0.7	0.03	0.4	0.9	0.0003	0.006
90	0.8	0.5	0.007	0.4	0.006	0.1	0.6	0.00007	0.007
105	0.9	0.04	0.0004	0.3	0.1	0.2	0.1	0.006	0.005
120	0.06	0.4	0.0003	0.5	0.02	0.3	0.4	0.00006	0.02
135	0.6	0.2	0.01	0.08	0.2	0.3	0.01	0.08	0.06
150	0.7	0.09	0.003	0.3	0.4	0.5	0.04	0.06	0.4
150	0.3	0.2	0.003	0.5	0.8	0.2	0.1	0.1	0.8
165	0.6	0.1	0.0003	0.3	0.5	0.12	0.03	0.09	0.8
180	0.5	0.3	0.004	0.3	0.8	0.09	0.02	0.4	1
195	0.3	0.2	0.006	0.4	0.9	0.4	0.05	0.05	0.3
210	0.06	0.6	0.09	0.3	0.2	0.4	0.5	0.03	0.02
225	0.04	0.9	0.2	0.9	0.0003	0.09	0.7	0.0002	0.009
240	0.03	0.6	0.02	0.6	0.0007	0.3	0.4	0.0001	0.02
255	0.007	0.7	0.01	0.8	0.009	0.06	0.3	0.0006	0.001
270	0.3	0.5	0.03	0.3	0.98	0.7	0.6	0.006	0.05
285	0.2	0.5	0.06	0.5	0.1	1	0.3	0.0002	0.007

This level was maintained until the end of the five minute recording period.

LA: Post-cut differences from baseline

F95 tended to be slightly above baseline for the majority of the post cut period. This difference was significant at 60, 135, 165, 180 and 285 seconds after cutting when F95 was 2.0% ($p<0.01$), 1.4% ($p<0.05$), 1.4% ($p<0.01$) and 1.9% ($p<0.05$) above baseline respectively. There was no clear pattern in these changes over time as demonstrated in the Con group.

NaturO: Post-cut differences from baseline

The response was somewhere between that of the LA and Con groups, being approximately half that of Con in magnitude, and did not reach a maximum level until more than 30 seconds after antler cutting. This was followed by a steady decline similar to that seen in the Con group, but commencing earlier and reaching baseline levels by a little over two minutes after cutting. Significant increases above baseline were seen from 15 seconds and were 2.6 ($p<0.05$), 4.2% ($p<0.001$), 2.9% ($p<0.05$), 2.9% ($p<0.01$), 2.8% ($p<0.01$), 2.4% ($p<0.01$) and 1.9% ($p<0.05$) at 15, 30, 45, 60, 75, 90, and 105 seconds respectively. From 135 to 210 seconds after cutting, F95 was not different from baseline. An increase of 1.6% ($p<0.05$), 1.5% ($p<0.01$), 1.7% ($p<0.05$) 1.5% ($p<0.01$) and 2.0% ($p<0.01$) was seen at 210, 225, 240, 255 and 285 seconds respectively.

Between treatment comparisons

There were significant differences between treatment groups in the magnitude of the response in F95 after cutting.

Con vs LA: Post-cut differences between groups

Differences between Con and LA followed a similar pattern to those of Con vs baseline. Con was greater than LA for the entire five minute period. This was statistically significant for all periods during the first two minutes ($p<0.001$). After this time Con was significantly greater than LA at 135 ($p<0.05$), 165 ($p<0.05$), 210 ($p<0.05$), 225 ($p<0.05$), 240 ($p<0.01$), and 255 ($p<0.001$) seconds after cutting.

Con vs NaturO: Post-cut differences between groups

Con was significantly greater than NaturO group for all except four of the 15 second intervals. These were at 30, 210, 225 and 285 seconds after cutting. Between 30 and 45 seconds after cutting, when F95 reached a peak in the NaturO group, there was no significant difference between Con and NaturO.

NaturO vs LA: Post-cut differences between groups

F95 was significantly higher in the NaturO group than the LA group between 30 and 45 seconds after cutting. There were no other significant differences between NaturO and LA after cutting.

Discussion

Please see the general discussion at the end of this chapter for a full discussion of the results and methods in this experiment.

Conclusions

Antler removal without analgesia resulted in significant changes in the EEG of anaesthetised deer. Application of a *NaturO*TM ring prior to antler removal reduced the EEG response to antler removal compared to that seen when no analgesia was applied prior to antler removal. Application of local anaesthetic prior to cutting prevented changes in EEG in response to antler cutting. *NaturO*TM ring did not reduce the EEG response to antler cutting as much as local anaesthetic.

For total power (TP) and 95% spectral edge frequency (F95) the response in the *NaturO*TM ring group was similar to that of the local anaesthetic group. For median frequency (F50) results in the *NaturO* were similar to the control group. Further research is required to determine the relationship between acute somatic noxious stimulus and the EEG response in different part of the frequency spectrum and the contribution of different pain fibre types.

3.2: Effects of lignocaine and *NaturO*TM rings on the behaviour of one-year-stags during velvet antler removal

Introduction

The aim of this experiment was to determine whether there was a difference in the behaviour of stags during velvet antler removal if they had been administered local anaesthetic or a *NaturO*TM ring prior to antler removal. Observation of a withdrawal response during velvet antler removal has been used in several studies (E.g. Matthew et al, 1992; Webster et al 2002, 2003; Wilson et al, 1999; 2000; Bartels et al, 2001; Woodbury et al 2001, 2002). As discussed in Chapter 1, the behavioural criteria used to determine a withdrawal response has only been defined in broad terms and there have not been any studies describing the differences in behaviour between adult and yearling stags. Most of the previous experiments have used electrical stimulation of the antler as a noxious stimulus rather than antler cutting. This removes some of the non-specific stimulus related to handling the head and saw vibration, but may not elicit the same pain response as actually cutting the antler with a saw. Chapter 2 of this thesis identified some more specific behaviours that indicated failure of local analgesia in adult stags. In the current experiment, a similar behaviour assessment was used for one-year-old stags, with an emphasis on recording behaviours as they were seen, during antler cutting, and in objective terms, using an ethogram approach.

Materials and Methods

Animals

60 rising-one-year-old male red deer were used. These were made up of animals born and reared on the Massey University Deer Unit and a cohort of animals bought in from a commercial farm and managed on the Massey University deer unit. The majority of deer were observed twice (once for each antler) resulting in 115 antler removal observations. Fifty four observations were from Massey reared animals and 61 were from bought in animals.

Treatments

A single-blinded, video-recorded experimental design comparing two treatments was used. Each antler removal was treated as a separate observation. Antlers were randomly assigned to one of two treatment groups:

- Lignocaine (Lig, n=49)

- *NaturO*TM ring (*NaturO*, n=66).

There were more animals in the *NaturO*TM ring group due to a requirement for these deer to be transported to slaughter immediately after velveting. Antlers were removed when they were longer than 110mm in length from the midline of the skull to the furthest extreme of the antler as this is the requirement for transport of yearling deer. All antlers were removed by one operator.

Procedure

1. Each deer was restrained in a hydraulic crush and handled as follows

Lig group

- Handled around the pedicle for 5 to 10 seconds

***NaturO* group**

- A *NaturO*TM ring applied using the cable-tied method (Deer Industry News, NVSB update).
2. The time was recorded and the deer released into a holding area.
 3. After approximately 55 minutes the deer was brought back into the hydraulic crush and treated as follows:

Lig group

- A rubber tourniquet was placed on the antler.
- Local anaesthetic (Lignocaine hydrochloride 2%, “Nopaine”, Phoenix Pharm.) was placed around base of the pedicle in a continuous ring, at a dose of 1mL per centimetre pedicle circumference.
- A black Velcro band was used to cover the site of lignocaine placement.

***NaturO* group**

- A black Velcro band was placed over the *NaturO*TM ring so that it would not be visible on video.
4. The antler was removed using a small pruning saw, not less than one hour after placement of the *NaturO*TM ring or between two and five minutes after application of the local anaesthetic. One blinded operator treated both groups.
 5. Animal tag number, antler (left or right) and time of antler removal were recorded on an Excel spreadsheet and on the video recording. Treatments were recorded against animal tag number and antler (left or right) on a separate sheet so treatment would not be evident during video analysis.

Video recordings:

Two Sony handycam digital video cameras (Model No. DCR-PC109E) recording onto miniDV

digital video tapes were operated.

Camera 1: Wide Angle

- This video camera was mounted above and to the front of the crush and fitted with a wide angle lens. The entire crush was visible.
- Filming was continuous during placement of the *NaturO*TM ring or local anaesthetic and also during removal of the antler.

Camera 2: Close up

A hand held video camera was used during velvet antler removal. This camera was focused on the head to record behavioural responses in detail.

Video analysis/Data collection.

Blinding

The person performing video analysis was blinded to treatment due to the Velcro bands placed over the ring block or *NaturO*TM ring prior to removal of the antler.

Behaviours recorded

The list of behaviours to be recorded was decided after initial viewing of a number of randomly selected video tapes. These included behaviour seen and described in previous observations of adult stags (Flint et al, 2005; Chapter 2, this thesis). In addition, novel behaviours were identified in these yearling stags. Video recordings were observed on a television screen and behaviours recorded onto Table 3.2.2. The treatment was not known at the time of behaviour recording due to the Velcro band on the antler pedicle.

Outcome classification

Behaviours demonstrated during antler removal were used to classify the animals into one of three groups: “Withdrawal”, indicative of perception of an acute noxious stimulus associated with saw cut; “No response”, absence of such behaviour; “Not Determinable”, a withdrawal response or lack thereof, can not be identified due to constant movement or behaviours associated with attempts to escape restraint.

In order to be classified as a “Withdrawal” the animal had to meet the criteria outlined in Table 3.2.4 (Modified from Chapter 2, this thesis).

Data analysis

Data were analysed using Microsoft Excel and Minitab 14 software. Estimates of the proportion of a population expected to demonstrate the outcome were made, based on sample proportion

and sample size. Comparisons between groups were made using two-sample proportion Z-tests.

Differences were considered significant when $p < 0.05$. Several of the comparisons involved counts of less than 5 and therefore the validity of the test is questionable. In these cases, Fisher's exact tests were performed as indicated. Even using this test, the results are questionable with low numbers (1 or 0) in some groups.

Results

Behaviours recorded

The behaviours that were recorded are described in Table 3.2.1.

A full list of the behaviours recorded for each animal is provided in Table 3.2.2 along with the source farm and treatment. (Note, these details were added after the observations were entered to ensure the observer was not biased during behavioural recordings). Based on the observed behaviours during antler removal each animal was classified as either showing a withdrawal response, no withdrawal response or not determinable according to the criteria in Tables 3.2.3 and 3.2.4.

Outcomes

Table 3.2.5 shows the number, proportion and predicted 95% confidence interval of antlers that were classified as "Withdrawal", "No Response" or "Not Determinable (ND)" for bought in stags and Massey-reared stags that were provided with either a NaturO™ ring or lignocaine for analgesia.

Table 3.2.6 shows statistical comparison for equal proportion between different groups and treatments.

Withdrawal response

Three animals were classified as showing a withdrawal response during antler cutting. Two of these had a NaturO™ ring for analgesia and one had subcutaneous lignocaine.

There were no significant differences between any groups in the proportion of animals that demonstrated a withdrawal response.

No response

Eighty seven (76%) of animals were classified as showing "no response" during antler cutting.

There were no significant differences between Lignocaine and NaturO. Significantly more brought in stags were classified as No Response compared to Massey-reared stags (93 vs 56% respectively). The 95% confidence of the difference between these groups is estimated to be 23 to 53%. This

difference is attributable to the large number of not-determinable outcomes in the Massey group.

Not determinable

There was no significant difference between *NaturO* and Lignocaine in the proportion of animals classified as Not Determinable.

Massey-reared animals were more likely to be classified as Not Determinable than Bought-in stags. (43 vs 3% respectively). The 95% confidence interval of the difference between these groups is estimated to be 25 to 53%.

Discussion

For discussion of the methods and results of this experiment, please see the general discussion at the end of this chapter

Conclusions

Behaviour produced confounding results that were difficult to interpret in relation to pain perception or failed analgesia in one-year-old red deer when a control (no analgesia) treatment could not be included for comparison.

There were marked differences in behavioural responses between cohorts of deer. No recommendations can be made regarding behaviour observations as an assessment of analgesic efficacy in rising one-year-old deer that can be applied across different farms.

Within the scope of this study there was no difference between *NaturO*TM rings and lignocaine on the withdrawal response of yearling deer during velvet antler cutting.

Table 3.2.1 Description of behaviours recorded during cutting the antlers of yearling stags that had either subcutaneous lignocaine or a NaturO™ ring applied for analgesia.

Abbreviation	Behaviour	Description
Ear	ears down	Sudden flattening of both ears
EF	eye flicker	Slight closing of the eyelids or blink
fl	flinch	Small, sharp rapid movement of the head with minimal displacement, usually in response to a stimulus
G2	Grade 2 move	Movement involving the whole head and neck not in any other category
Hdip	Head dip	Lowering of the head preceding another movement, usually a NU
HF	Head flick	Abrupt sideways or upwards movement of the whole head
HR	Head rotate	Ventral displacement of one antler and dorsal displacement of the other antler
HTw	Head twist	Movement of the whole head that includes sideways and rotational movement.
LA	Looking around	Orientation of eyes and ears towards external stimulus
Launch	Launch	Single movement of the whole body in an abrupt forward direction
NU	Nose up	Scooping movement of the nose in an upwards and backwards arc
Str	Struggle	Whole body movements attempting to escape
TF	Tongue flick	Flicking the tongue out the side of the mouth

Table 3.2.2 Behaviours recorded from individual yearling stags during antler removal following application of either subcutaneous LANocaine or a NaturO™ ring. Outcome shown as either withdrawal, no response or not determinable. Farm of orAin M=Massey, B= brought in.

Ob	OrAin	reactions	Comments	With- drawal	No Response	Not Determinable	TREAT
1	M	LA, LA			✓		NO
2	M	LA, HF, HR, HR	Not repeated after initial cut, being difficult			✓	NO
3	M	slow Hdip, NU start saw. Hdip, NU, EF.	Turning head a lot. EF but saw was banging against ear			✓	NO
4	M	HU at end	Some flickering vibrations in time with saw cuts			✓	NO
5	M	flinch, HD, HA, HR not repeated	Moves at start only		✓		NO
6	M	LA			✓		LA
7	M	NU, NU, Launch (but hardly touched with saw), Str, fl, NU, LA, Str	More local given but still NU, LA and Str			✓	LA
8	M	NU	slight moves		✓		LA
9	M	LA, NU, HR, slow HR, LA, Launch				✓	LA
10	M	Small LA, LA, LA	Struggling and moving repeatedly before cutting		✓		LA
11	M				✓		LA
12	M	NU first touch, HU, difficult	Str, Launch before cut, being very difficult during cutting			✓	LA
13	M	HU HR NU NU G2 G2 HTw	HA and HR before cutting, being difficult			✓	LA
14	M	HA, HR, TF, EF, Str, NU				✓	NO
15	M	NU, Str, TF, LA, NU, HR, Str Ear		✓			NO
16	M	HA then constantly wrA- gling before, during and after cutting	ND, NU, HU jumpy before start cutting, similAr HU movements and LA throughout cutting, being difficult			✓	NO
17	M	nU HF, HF sideways, NU, NU, NU	Tried towel over eyes, no change in behaviour			✓	NO
18	M	NU, Str, TF, HR, NU, HD	Generally struggling a lot throughout			✓	LA
19	M				✓		NO
20	M	fl, NU, NU, NU, HR, TF, Str	Moving constantly throughout			✓	LA
21	M	slight HA	jumping around and flicking tongue prior to cut		✓		LA
22	M				✓		LA
23	M	HF at end			✓		LA
24	M				✓		LA
25	M		Stressed and growling before cut, no move during, holding nose		✓		NO
26	M	LA, HR, HF, NU				✓	NO

Table 3.2.2 cont.

Ob	OrAin	reactions	Comments	With-drawal	No Response	Not Determinable	TREAT
27	M				✓		NO
28	M	small slow HA and LA			✓		NO
29	M	HU, waving head in th air throughout, HF at end	moving head around, difficult			✓	NO
30	M	NU	Held nose down, no further movements		✓		NO
31	M	HA, NU, TF				✓	NO
32	M				✓		NO
33	M	small HF, small HF			✓		NO
34	M	LA, LA, LA			✓		NO
35	M	LA				✓	NO
36	M	NU at end				✓	NO
37	M	Str	big struggle but saw pulling back on antler got stuck, ok when cut from other direction			✓	NO
38	M	fl, HA, Str LA, fl HR Str.	Moving around, struggling throughout.			✓	NO
39	M	Fidget at start, then settled.			✓		NO
40	M					✓	NO
41	B				✓		NO
42	B				✓		NO
43	B	Str, Str, NU.	G3 G3 nu, similar moves before and during cut		✓		NO
44	B				✓		LA
45	B				✓		LA
46	B				✓		LA
47	B				✓		NO
48	B				✓		LA
49	B	eye wideing, slight HR.	cutting antler downwards	✓			NO
50	B				✓		LA
51	B	nothing			✓		NO
52	B	nothing			✓		LA
53	B	nothing			✓		NO
54	B	nothing			✓		NO
55	B	slight blinking			✓		LA
56	B	nothing			✓		NO
57	B	nothing			✓		NO
58	B	nothing			✓		LA
59	B	nothing			✓		LA
60	B	nothing			✓		LA
61	B	nothing			✓		NO
62	B	TF, TF, TF, poss flinches	difficult to see video		✓		NO
63	B	nothing			✓		LA
64	M	NU, NU, LA			✓		LA
65	M		Str before start		✓		NO

Table 3.2.2 cont.

Ob	OrAin	reactions	Comments	With- drawal	No Response	Not Determinable	TREAT
66	B	nothing			✓		NO
67	B	nothing			✓		LA
68	B	nothing			✓		NO
69	M	NU, LA HU LA NU	moving around nose up being difficult, antler hard		✓		NO
70	B	nothing			✓		LA
71	B	nothing			✓		NO
72	B	nothing			✓		NO
73	B	nothing			✓		LA
74	M	HU ears	lifting head, awkward		✓		LA
75	M	nothing			✓		NO
76	B	violent Str NU, TF, once only, not repeated	made similar movements prior to cutting			✓	NO
77	B	Str, launch TF near end of cutting at back	made similar movements prior to cutting			✓	LA
78	B	nothing			✓		LA
79	B	v slight HU			✓		NO
80	B	nothing			✓		NO
81	B	nothing			✓		LA
82	B	nothing			✓		LA
83	B	nothing			✓		NO
84	M	NU, LA, wriggling, moving constantly	constant movements, being difficult		✓		NO
85	M	LA			✓		LA
86	B	Growl, snap at fly			✓		NO
87	B	nothing			✓		LA
88	B	LA			✓		NO
89	B	nothing			✓		LA
90	B	slow HD, LA	looking around a bit, noisy in shed		✓		LA
91	B	nothing			✓		NO
92	B				✓		NO
93	B	small HU, squint eye			✓		LA
94	B	nothing			✓		NO
95	B	nothing			✓		LA
96	M	Throwing head all over the place	very difficult animal			✓	NO
97	M	Constant movements during restraint	Difficult animal			✓	LA
98	B	nothing			✓		LA
99	B	nothing			✓		NO
100	M	violent HU	cut ear when head thrown up		✓		NO
101	M	nothing			✓		LA
102	B	nothing			✓		LA
103	B	nothing			✓		NO
104	B	HU	saw moving head back and forth		✓		NO

Table 3.2.2 cont.

Ob	OrAin	reactions	Comments	With- drawal	No Response	Not Determinable	TREAT
105	B	nothing			✓		NO
106	B	nothing			✓		LA
107	B	HA, G2, possible flinches	difficult to see	✓			LA
108	B	G2, G2, possible flinches	Similar moves as seen with other antler cutting		✓		NO
109	B	nothing			✓		NO
110	B	nothing			✓		LA
111	M	HU, LA, LA	slow movements		✓		NO
112	B	nothing			✓		NO
113	B	nothing			✓		LA
114	M	nothing	throwing head around before started, nothing during cut		✓		LA
115	M	moving a lot during cut	being difficult before cutting			✓	NO
				3	87	25	

Table 3.2.3 Criteria for determining outcome classifications from reactions during velvet removal from yearling stags

Classification	Criteria
Withdrawal	Criteria in Table 3.2.4 met
No Response	Absence of withdrawal behaviour
Not Determinable	Background movement or behaviour does not allow discrimination of criteria for a withdrawal response

Table 3.2.4 Requirements for classification of a withdrawal response to velvet antler cutting in yearling stags

Compulsory requirements (both):	
	Behaviour must occur at the same time as potentially painful saw pressure
	Behaviour must occur more than once during cutting. (Exception below ¹)
Additional requirements (any one or more of)	
	Flinch
	Head arch, particularly if repeated or associated with a flinch
	Directional movement away from the stimulus (down or sideways)
	Repeated movements while cutting the same antler section ²
	Behaviours seen only during cutting and not during other handling periods ²
Modifications / Exceptions	
1	A single behaviour may be classified as a withdrawal response when it meets all other criteria and the animal has not demonstrated any movements during handling, except at the time of antler cutting.
2	Whole body struggles and gross movements are considered “non-specific” behaviours. If these behaviours are seen during antler cutting only and not at any other time during handling, they may be classified as withdrawal provided all other criteria are met.

Table 3.2.5 Proportion of observed response (number of animals, n, in parenthesis) and 95%CI (in parenthesis below) that were classified as either a withdrawal response, no response or not determinable (ND) behavioural outcomes during antler cutting after either subcutaneous lignocaine or a NaturO™ ring for analgesia. Deer were sourced either on the Massey farm or brought in as shown.

Source	Treatment	n	ND	Behavioural outcome No Response	Withdrawal
Brought in	Lig	29	0.03 (n=1) (0.00 to 0.09)	0.93 (n=27) (0.83 to 1.00)	0.03 (n=1) (0.00 to 0.09)
	NO	32	0.03 (n=1) (0.00 to 0.09)	0.94 (n=30) (0.86 to 1.00)	0.03 (n=1) (0.00 to 0.09)
	Total	61	0.03 (n=2) (0.00 to 0.07)	0.93 (n=57) (0.87 to 0.99)	0.03 (n=2) (0.00 to 0.07)
Massey	Lig	20	0.35 (n=7) (0.13 to 0.57)	0.65 (n=13) (0.43 to 0.87)	0.00 (n=0) (0)
	NO	34	0.47 (n=16) (0.29 to 0.65)	0.50 (n=17) (0.32 to 0.68)	0.03 (n=1) (0.00 to 0.09)
	Total	54	0.43 (n=23) (0.29 to 0.57)	0.56 (n=30) (0.42 to 0.70)	0.02 (n=1) (0.00 to 0.06)
Combined (Massey + brought in)	Lig	49	0.16 (n=8) (0.06 to 0.26)	0.82 (n=40) (0.72 to 0.92)	0.02 (n=1) (0.00 to 0.06)
	OR	64	0.26 (n=17) (0.16 to 0.36)	0.71 (n=47) (0.59 to 0.83)	0.03 (n=2) (0.00 to 0.07)
	Total	115	0.22 (n=25) (0.14 to 0.30)	0.76 (n=87) (0.68 to 0.84)	0.03 (n=3) (0.00 to 0.07)

Table 3.2.6 Statistical test results for equal means in predicted behavioural outcome during antler cutting of yearling stags from two different sources that were treated with either lignocaine local anaesthetic (LA) or a NaturO™ ring (NO) for analgesia

Test	sample	Est	95% CI of dif-			Fisher's exact	Mark if Ho
Samples	n	proportion	ference	Z	p-value	test p-value	rejected
Withdrawal							
Massey NO	34	0.00					
vs Massey LA	20	0.029	-0.029	-0.086, 0.027	-1.02	0.310	1.0
Bought NO	32	0.031					
vs Bought LA	29	0.034	0.003	-0.086, 0.093	0.07	0.944	1.0
Massey tot	54	0.019					
vs Bought tot	61	0.033	0.014	-0.043, 0.072	0.49	0.626	1.0
Combined NO	66	0.030					
vs Combined LA	49	0.020	-0.0099	-0.067, 0.053	-0.34	0.735	1.0
No Response							
Massey NO	34	0.50					
vs Massey LA	20	0.65	0.15	-0.12, 0.42	1.10	0.273	
Bought NO	32	0.94					
vs Bought LA	29	0.93	0.01	-0.13, 0.12	-0.10	0.919	1.0
Massey tot	54	0.56					
vs Bought tot	61	0.93	0.38	0.23, 0.53	5.07	<0.001	✓
Combined NO	66	0.71					
vs Combined LA	49	0.82	0.10	-0.05, 0.26	1.33	0.184	
Not Determinable							
Massey LA	20	0.35					
vs Massey NO	34	0.47	-0.12	-0.39, 0.15	-0.88	0.378	
Bought LA	29	0.034					
vs Bought NO	32	0.031	0.003	-0.086, 0.093	0.07	0.944	1.0
Massey total	54	0.43					
vs Bought total	61	0.03	-0.39	-0.53, -0.25	-5.53	<0.001	✓
Comb LA	49	0.16					
vs Comb NO	66	0.26	-0.094	-0.242, 0.053	-1.25	0.211	

3.3 Effect of application of lignocaine or *NaturO*TM rings on the EEG spectrum of anaesthetised one-year-old red deer.

This is the first of two experiments examining whether *NaturO*TM rings may be painful when they are applied to the pedicle of yearling stags. The methods used are similar to those used in experiment 3.1 except that the EEG recordings were made during application *NaturO*TM ring and local anaesthetic rather than during antler cutting. There was no control group in this study. The use of EEG is discussed in Chapter 1.2.2. This is the first experiment that we are aware of that has tested the noxiousness of the *NaturO*TM ring in yearling deer and is similar to the experiment conducted by Johnson et al (2005) investigating the noxiousness of high pressure compression bands on adult stags.

Methods and Materials

Animals

Twenty rising one-year-old male red deer weighing 78.2 ± 6.1 kg (SD) were used. Deer were brought onto the property on 17 August 2007 and the experimental procedures were conducted between October 2007 and January 2008.

Treatments

The treatments were:

- Lignocaine (Lig)
- *NaturO*TM ring (*NaturO*)

Animals were assigned alternately to a treatment, so that the first deer had local anaesthetic, the second had *NaturO*TM rings, etc. Experiments were conducted on each animal as soon as the antlers were long enough to place a *NaturO*TM ring. So that the first deer to develop his antlers was the first to undergo experimentation and was treated with LA, the second to develop his antlers was treated with *NaturO* etc.

Lignocaine

Local anaesthetic (Lignocaine hydrochloride 2%, “Nopaine”, Phoenix Pharm.) was placed around base of the pedicle in a continuous ring, at a dose of 1mL per centimetre pedicle circumference.

*NaturO*TM ring

A standard *NaturO*TM Ring (AgResearch) was applied to both antlers using the tied method

(Farmer Velvet Antler Removal Manual) (Nicol et al, 2011).

Antlers were not removed in this experiment.

Anaesthetic protocol

The anaesthetic protocol was the same as that used in Chapter 3.1.

EEG recording procedure.

The setup of EEG recording equipment was the same as in Chapter 3.1. EEG recorded commenced not less than 30 minutes after induction of general anaesthesia and after the animal was stabilised at an end tidal halothane concentration of 0.9%. Baseline (pre-treatment) recordings were made for five minutes prior to application of the treatment and post-treatment recordings were made for ten minutes after the treatment was finished. Two channels of EEG were recorded, being from the left and right cerebral hemispheres. These two channels of data were analysed separately.

Data Analysis

Inspection of Raw Data

Raw EEG was inspected visually for artefacts. The raw EEG recordings were then cut to 300 seconds before treatment to 600 seconds after treatment. Time of treatment was taken as the time that it started. Applying the treatment took between 10 and 15 seconds for both antlers.

The cut EEG segments were then converted to a frequency spectrum by Fast Fourier Transformation (Welch, 1967) using software developed by Craig Johnson (FFT program, Craig Johnson). Frequency band amplitudes were determined in one second epochs from 1000 samples per second from the original sampling. The median frequency (F50), 95% spectral edge frequency (F95) and total power (TP) of the spectrum in each second were calculated and these data were analysed further for comparison of animals and treatments.

Baseline calculations

In order to compare treatment groups, it was necessary to standardise the data to percentage of baseline. The average of the two minutes prior to applying the treatment was chosen as a representative baseline. All one-second, 15-second or one-minute means were then divided by the baseline average and multiplied by 100. Thus baseline was corrected to 100% for all deer and each time interval was recorded as percent of the two-minute pre-treatment baseline. Subjects within each treatment group could then be grouped and the mean and standard deviation calculated.

Statistical analysis

Data were analysed in both 15 second and 1 minute average time intervals. Different time inter-

vals were explored to see whether different patterns in the data could be identified.

Data was grouped by treatment, Lignocaine (Lig) or *NaturO*TM ring (*NaturO*), and TP, F95 and F50 were analysed separately. Two-way ANOVA was performed using Prism 4 software to assess the effects of treatment and time and the interaction between treatment and time. Where ANOVA indicated that treatment had a significant effect, Bonferroni post-test comparisons of the mean were done. Where time was found to have a significant effect, student t-test was performed for each time interval compared to baseline. Differences were considered significant when $p < 0.01$. This p -value was chosen because it increased the specificity of the test and reduced random noise in the results compared to using a p -value of 0.05.

Results

Visual inspection of data

Figures 3.3.1a and b show the F50, F95 and TP from both channels of individual animals. The graphs on the first page (Fig 3.3.1a) show animals that were treated with a lignocaine ring block and the graphs on the second page (3.3.1b) show animals that were treated with a *NaturO*TM ring. Each animal is plotted on a separate panel. Plotted points are the average of the 15-second interval before the time indicated on the X-axis.

In all animals except deer 10 the values and pattern of change over time for F50 and F95 were similar in the left and right channels for individual animals. One channel of data (Deer 10, channel 1) was excluded from further analysis due to high and variable total power recordings. Although the F50 and F95 were affected to a lesser degree it was decided to discard these data from analysis. Channel 2 from the same animal contained normal data patterns indicating a problem with the electrode on one side of the animal only.

Graphical representation of the data for each treatment over time

Time series plots are presented in the following average time intervals:

- Averages for each second (Figure 3.3.2)
- Averages for each 15 second period (Figure 3.3.3)
- Averages for each one minute period (Figure 3.3.4)

Figures 3.3.2 and 3.3.3 are plotted from two minutes prior to treatment until five minutes after treatment and figure 3.3.4 is plotted from five minutes prior to treatment until ten minutes after treatment. For the 15 second and one minute averages, error bars show the 95% confidence interval of the mean and symbols indicate t-test results of $p < 0.01$ (99% probability of statistically significant difference between groups).

Two way ANOVA

Two-way ANOVA on one minute averages from 5-minutes before treatment to 10 minutes after

treatment and 15-second averages for two minutes before treatment until 5 minutes after treatment are shown in Table 3.3.1. There were significant interactions between treatment and time for all variables. This suggests that effects were different over time between the two treatments. Both treatment and time had a significant effect on TP. Treatment has a significant effect on F95, whereas time did not significantly effect F95 overall in the two-way ANOVA. When ANOVA was run with repeated measures, time had a significant effect ($p < 0.001$) on F95, therefore baseline comparisons using T-tests were performed for each time interval within each treatment group. For F50, time had a significant effect whereas treatment had less or no effect. Treatment had a significant effect on F50 when one-minute averages were compared, therefore T-test were performed to test the difference between treatments at each time interval.

Bonferroni post-test comparisons between treatments at each time interval for both one-minute and 15-second data are shown in Table 3.3.2 and Table 3.3.3, respectively. Student T-test comparisons within each treatment comparing each time interval with baseline recording are presented in Table 3.3.4 and Table 3.3.5 for 1-minute and 15-second averages, respectively.

Baseline stability

There were very few significant differences ($p > 0.01$) between treatments or within treatments over time in baseline recordings for TP, F50 or F95.

Total Power (TP)

Lignocaine vs Baseline

In the lignocaine group, TP increased steadily until five minutes after treatment and then remained constant and significantly elevated until the end of recording at ten minutes after treatment.

NaturO vs Baseline

NaturO™ rings caused an initial increase in total power during the first 30 seconds. This was followed by a decrease during the second minute after treatment ($p < 0.01$). These were the only changes seen in TP.

Figure 3.3.1 (overleaf) Total power, median frequency and spectral edge frequency averaged over 15-second intervals for the period from five minutes before treatment and ten minutes after treatment with either lignocaine ring block at the base of both antler pedicles (a) or a NaturO™ ring to both antler pedicles (b). Each graph panel represents an individual animal and both left and right channels are shown on the same graph. Time is shown on the x-axis with the time of treatment indicated with a solid black line. The y-axis shows power for TP and frequency (Hz) for F50 and F95.



3.3.1a Lignocaine (see previous page for caption)



3.3.1b NaturO™ ring

Table 3.3.1 Summary of Two-way ANOVAs for effects of time, treatment and interaction on TP, F50 and F95 from electroencephalogram recordings from yearling male deer that were treated with either a lignocaine ring block at the base of the antler or a NaturO™ ring. Calculations are shown for averages taken over each minute from five minutes prior to treatment until ten minutes after treatment and for average taken over each 15 second interval from 2 minutes prior to treatment until five minutes after treatment. p-value is the probability that these results would have occurred if treatment, time or interaction had no effect.

	Treatment	Time	Interaction
TP			
1-Minute averages	yes (p<0.0001)	yes (p<0.0001)	yes (p<0.0001)
15-second averages	yes (p<0.0001)	yes (p=0.0063)	yes (p<0.0001)
F50			
1-Minute averages	yes (p=0.0016)	yes (p<0.0001)	yes (p<0.0001)
15-second averages	no (p=0.82)	yes (p<0.0001)	yes (p<0.0001)
F95			
1-Minute averages	yes (p<0.0001)	no (p=0.099)	yes (p<0.0001)
15-second averages	yes (p<0.0001)	no (p=0.013)	yes (p<0.0001)

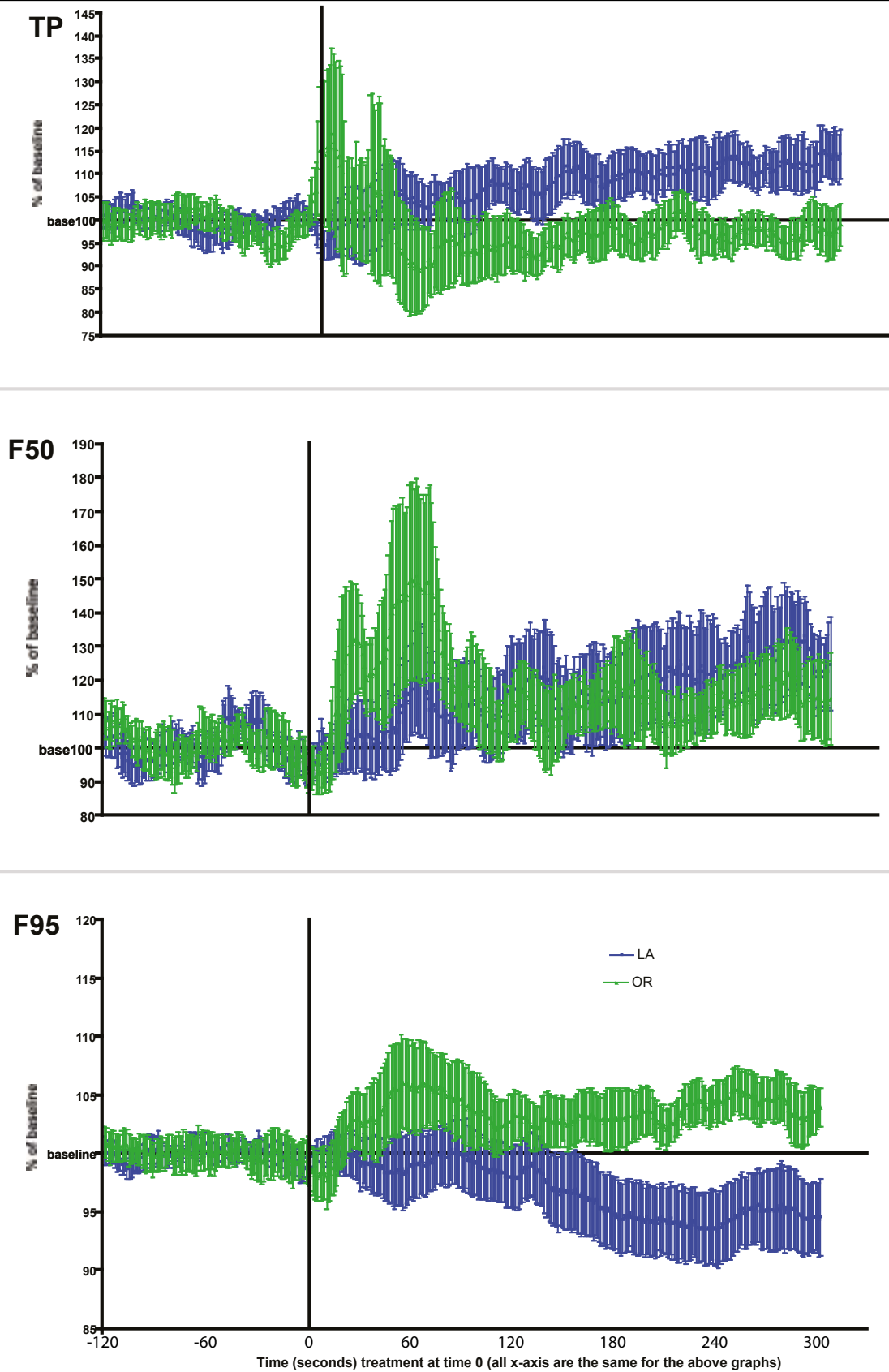


Figure 3.3.2 Moving average of 15 second means plotted every second for TP, F50 and F95 from encephalogram recording from yearling deer that were treated with either a lignocaine ring block (blue) or NaturO™ ring (green). Time is from -2 minutes to +5 minutes from treatment. Treatment time is indicated by the solid black line

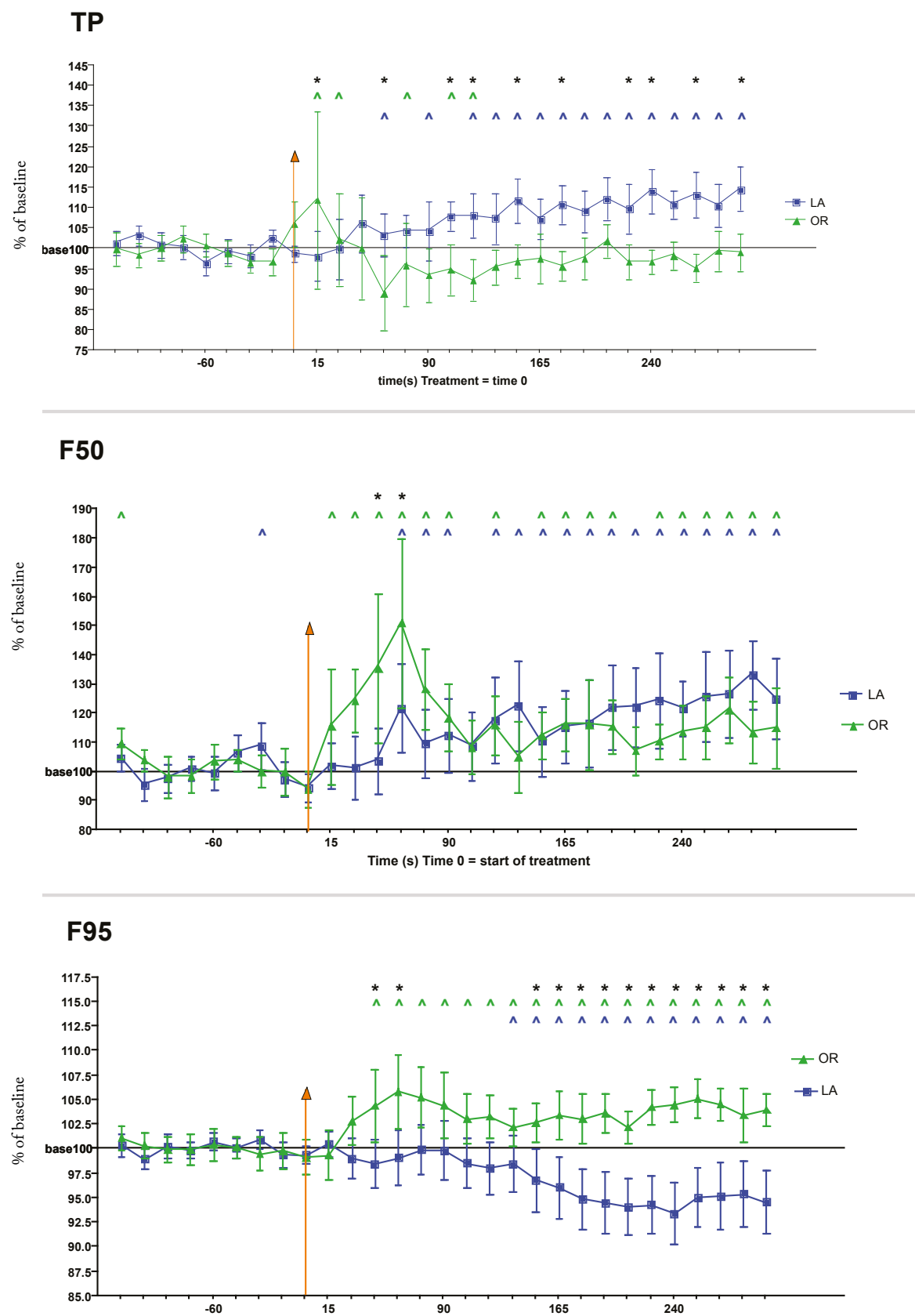
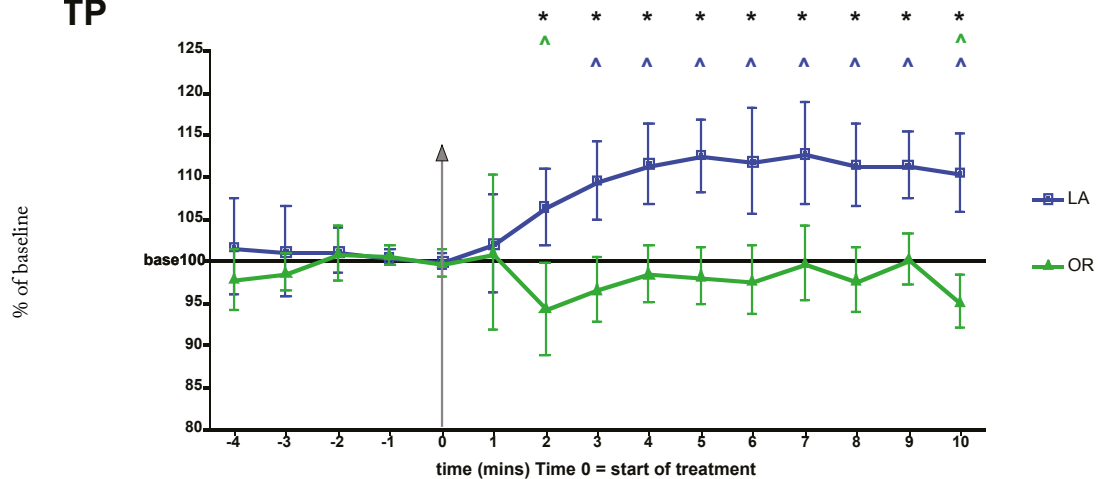
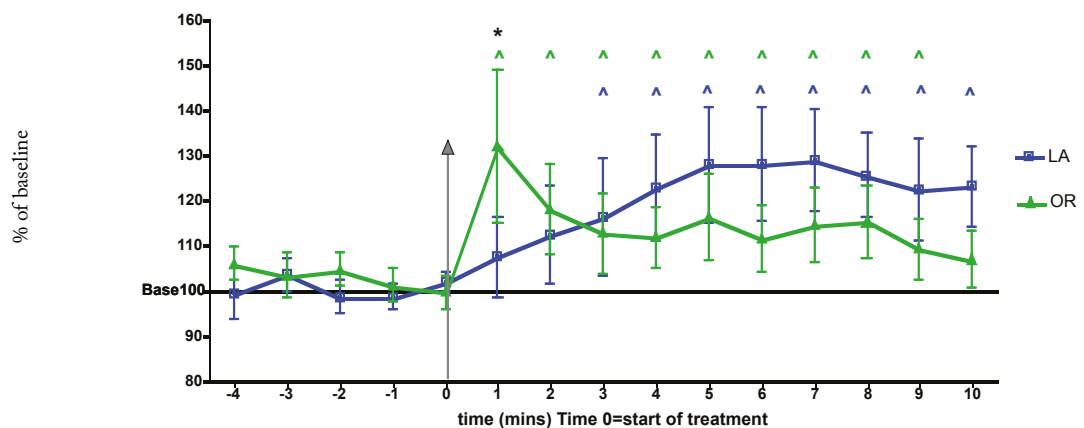


Figure 3.3.3 Mean TP, F50 and F95 of the electroencephalogram recorded from yearling deer that had either a lignocaine ring block administered (blue) or a NaturO™ ring applied (green) Error bars are 95% confidence intervals. Time of recording is from two minutes prior to treatment until five minutes after treatment and averaged over 15 second intervals. Arrow indicates the time of treatment. Significant differences are indicated above each graph ^ = significantly different from baseline within each group, * = significant difference between treatment groups

TP



F50



F95

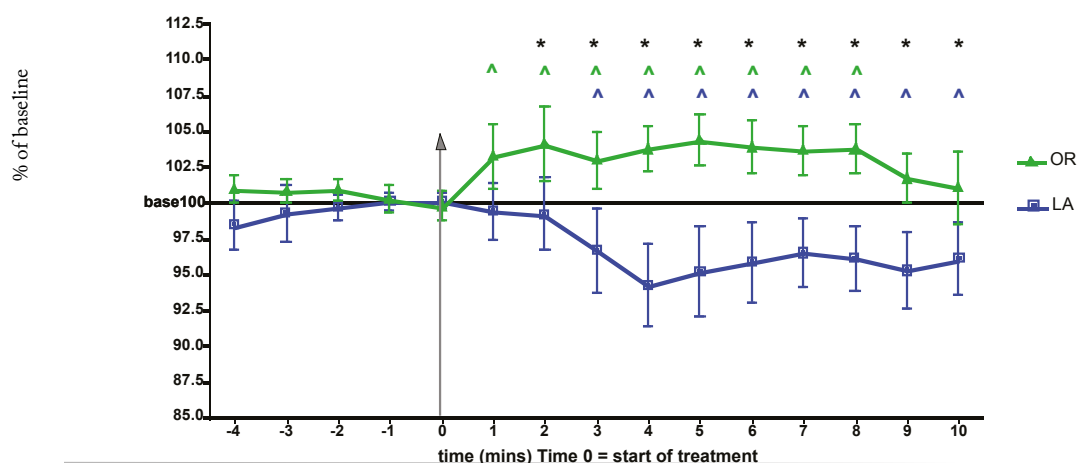


Figure 3.3.4 Mean TP, F50 and F95 of the electroencephalogram recorded from yearling deer that had either a lignocaine ring block administered (blue) or a NaturO™ ring applied (green). Error bars are 95% confidence intervals. Time of recording is from five minutes prior to treatment until ten minutes after treatment and averaged over one minute intervals. Arrow indicates the time of treatment. Significant differences are indicated above each graph ^ = significantly different from baseline within each group, * = significant difference between treatment groups

Table 3.3.2 Post ANOVA Bonferroni comparisons of Total Power, Median Frequency and 95% spectral edge frequency from electroencephalogram recordings from deer that were treated with either a lignocaine ring block or a NaturO™ ring. Difference shown is Lignocaine – NaturO. Comparisons are for each one minute interval from 5 mins prior to treatment until 10 mins after treatment.

	Time (mins)	Difference	t	P value	Summary
TP	-4	-3.815	1.279	P > 0.05	ns
	-3	-2.527	0.8474	P > 0.05	ns
	-2	-0.2931	0.09829	P > 0.05	ns
	-1	0.2340	0.07845	P > 0.05	ns
	0	-0.1807	0.06059	P > 0.05	ns
	1	-1.051	0.3523	P > 0.05	ns
	2	-12.10	4.058	P<0.001	***
	3	-12.91	4.329	P<0.001	***
	4	-13.02	4.367	P<0.001	***
	5	-14.31	4.797	P<0.001	***
	6	-14.22	4.770	P<0.001	***
	7	-13.10	4.392	P<0.001	***
	8	-13.64	4.574	P<0.001	***
	9	-11.30	3.789	P<0.01	**
	10	-15.39	5.161	P<0.001	***
	time	Difference	t	P value	Summary
F50	-4	6.771	1.143	P > 0.05	ns
	-3	-0.1845	0.03114	P > 0.05	ns
	-2	6.011	1.015	P > 0.05	ns
	-1	2.594	0.4378	P > 0.05	ns
	0	-2.328	0.3928	P > 0.05	ns
	1	24.40	4.118	P<0.001	***
	2	5.671	0.9571	P > 0.05	ns
	3	-3.715	0.6271	P > 0.05	ns
	4	-10.97	1.852	P > 0.05	ns
	5	-11.47	1.937	P > 0.05	ns
	6	-16.62	2.804	P > 0.05	ns
	7	-14.12	2.384	P > 0.05	ns
	8	-10.27	1.733	P > 0.05	ns
	9	-13.23	2.234	P > 0.05	ns
	10	-16.11	2.719	P > 0.05	ns
	time	Difference	t	P value	Summary
F95	-4	2.531	1.838	P > 0.05	ns
	-3	1.518	1.102	P > 0.05	ns
	-2	1.266	0.9193	P > 0.05	ns
	-1	0.215	0.1566	P > 0.05	ns
	0	-0.304	0.2209	P > 0.05	ns
	1	3.844	2.791	P > 0.05	ns
	2	4.923	3.575	P<0.01	**
	3	6.288	4.566	P<0.001	***
	4	9.574	6.952	P<0.001	***
	5	9.232	6.703	P<0.001	***
	6	8.049	5.845	P<0.001	***
	7	7.113	5.165	P<0.001	***
	8	7.652	5.556	P<0.001	***
	9	6.428	4.667	P<0.001	***
	10	4.973	3.611	P<0.01	**

Table 3.3.3 Post ANOVA Bonferroni comparisons of Total Power, Median Frequency and 95% spectral edge frequency from electroencephalogram recordings from deer that were treated with either a lignocaine ring block or a NaturO™ ring. Difference shown is Lignocaine - NaturO. Comparisons are for each fifteen second interval from 5 mins prior to treatment until 10 mins after treatment.

	Time	Diff	t	P value	Summary
TP	-120	-1.35	0.3378	P > 0.05	ns
	-105	-4.77	1.193	P > 0.05	ns
	-90	-0.69	0.1734	P > 0.05	ns
	-75	2.07	0.5199	P > 0.05	ns
	-60	4.31	1.079	P > 0.05	ns
	-45	-0.52	0.1321	P > 0.05	ns
	-30	-1.52	0.3812	P > 0.05	ns
	-15	-5.8	1.460	P > 0.05	ns
	0	7.1	1.791	P > 0.05	ns
	15	13.8	3.454	P < 0.05	*
	30	2.36	0.5916	P > 0.05	ns
	45	-6.18	1.546	P > 0.05	ns
	60	-14.2	3.552	P < 0.05	*
	75	-8.3	2.083	P > 0.05	ns
	90	-11.0	2.756	P > 0.05	ns
	105	-13.2	3.306	P < 0.05	*
	120	-15.8	3.961	P < 0.01	**
	135	-11.8	2.968	P > 0.05	ns
	150	-14.9	3.743	P < 0.01	**
	165	-9.6	2.417	P > 0.05	ns
	180	-15.1	3.785	P < 0.01	**
	195	-11.3	2.847	P > 0.05	ns
	210	-10.4	2.613	P > 0.05	ns
	225	-12.9	3.227	P < 0.05	*
	240	-17.3	4.336	P < 0.001	***
	255	-12.5	3.134	P > 0.05	ns
	270	-18.0	4.503	P < 0.001	***
	285	-11.2	2.799	P > 0.05	ns
	300	-15.5	3.874	P < 0.01	**

	Time	Diff	t	P value	Summary
F95	-120	0.78	0.4939	P > 0.05	ns
	-105	1.39	0.8775	P > 0.05	ns
	-90	-0.28	0.1823	P > 0.05	ns
	-75	0.09	0.06087	P > 0.05	ns
	-60	-0.33	0.2112	P > 0.05	ns
	-45	0.043	0.02720	P > 0.05	ns
	-30	-1.44	0.9099	P > 0.05	ns
	-15	0.40	0.2529	P > 0.05	ns
	0	-0.22	0.1409	P > 0.05	ns
	15	-1.11	0.7007	P > 0.05	ns
	30	3.79	2.388	P > 0.05	ns
	45	5.90	3.719	P < 0.01	**
	60	6.79	4.280	P < 0.001	***
	75	5.35	3.373	P < 0.05	*
	90	4.54	2.864	P > 0.05	ns
	105	4.51	2.840	P > 0.05	ns
	120	5.28	3.329	P < 0.05	*
	135	3.71	2.339	P > 0.05	ns
	150	5.87	3.701	P < 0.01	**
	165	7.37	4.645	P < 0.001	***
	180	8.18	5.158	P < 0.001	***
	195	9.15	5.770	P < 0.001	***
	210	8.10	5.108	P < 0.001	***
	225	9.96	6.276	P < 0.001	***
	240	11.07	6.973	P < 0.001	***
	255	10.1	6.375	P < 0.001	***
	270	9.38	5.915	P < 0.001	***
	285	8.02	5.056	P < 0.001	***
	300	9.38	5.914	P < 0.001	***

	Time	Diff	t	P value	Summary
F50	-120	4.71	0.6197	P > 0.05	ns
	-105	8.39	1.103	P > 0.05	ns
	-90	0.53	0.07075	P > 0.05	ns
	-75	-2.67	0.3508	P > 0.05	ns
	-60	4.10	0.5397	P > 0.05	ns
	-45	-2.50	0.3290	P > 0.05	ns
	-30	-8.74	1.149	P > 0.05	ns
	-15	2.47	0.3251	P > 0.05	ns
	0	-0.53	0.06963	P > 0.05	ns
	15	13.5	1.784	P > 0.05	ns
	30	23.12	3.037	P > 0.05	ns
	45	31.79	4.175	P < 0.01	**
	60	29.10	3.822	P < 0.01	**
	75	18.74	2.461	P > 0.05	ns
	90	6.08	0.7994	P > 0.05	ns
	105	-0.36	0.04753	P > 0.05	ns
	120	-1.7	0.2324	P > 0.05	ns
	135	-17.4	2.288	P > 0.05	ns
	150	1.89	0.2493	P > 0.05	ns
	165	0.94	0.1236	P > 0.05	ns
	180	-0.28	0.03750	P > 0.05	ns
	195	-6.6	0.8744	P > 0.05	ns
	210	-15.0	1.970	P > 0.05	ns
	225	-14.1	1.852	P > 0.05	ns
	240	-8.14	1.069	P > 0.05	ns
	255	-10.5	1.388	P > 0.05	ns
	270	-5.39	0.7080	P > 0.05	ns
	285	-19.7	2.591	P > 0.05	ns
	300	-10.2	1.341	P > 0.05	ns

Table 3.3.4 Summary of t-tests performed to compare post treatment measurements with pre-treatment baseline measurements of Total Power, Median Frequency and 95% spectral edge frequency from electroencephalogram recording from rising one-year-old male deer that were treated with either a lignocaine ring block at the base of the antler or a NaturO™ ring. Tests were done on averages for each one minute time period from five minutes prior to treatment until ten minutes post treatment. Lignocaine and O Ring results are shown side by side for each of the variables measured.

** p<0.01

*** p<0.001

ns: Not significant

3.3.4a Total Power differences from baseline for each minute before and after treatment.

Lignocaine

O Ring

TP						TP					
	time	mean	difference	p	summary		time	mean	difference	p	summary
	-4	102	2	0.4572	ns		-4	98	-2	0.0945	ns
	-3	101	1	0.9469	ns		-3	99	-1	0.1166	ns
	-2	101	1	0.3352	ns		-2	101	1	0.6099	ns
	-1	100	0	0.6668	ns		-1	101	1	0.5674	ns
	0	100	0	0.6721	ns		0	100	0	0.6086	ns
	1	102	2	0.3594	ns		1	101	1	0.8083	ns
	2	106	6	0.0002	***		2	94	-6	0.0035	**
	3	109	9	0.0000	***		3	96	-4	0.0172	ns
	4	111	11	0.0000	***		4	98	-2	0.1885	ns
	5	112	12	0.0000	***		5	98	-2	0.1260	ns
	6	112	12	0.0000	***		6	98	-2	0.0974	ns
	7	113	13	0.0000	***		7	100	0	0.7393	ns
	8	111	11	0.0000	***		8	98	-2	0.0856	ns
	9	111	11	0.0000	***		9	100	0	0.9665	ns
	10	110	10	0.0000	***		10	95	-5	0.0001	***

3.3.4b: Median Frequency differences from baseline for each minute before and after treatment.

Lignocaine						O Ring					
F50						F50					
	time	mean	difference	p	summary		time	mean	difference	p	summary
	-4	99	-1	0.3323	ns		-4	106	6	0.0122	ns
	-3	103	3	0.3732	ns		-3	103	3	0.2277	ns
	-2	99	-1	0.1871	ns		-2	105	5	0.0518	ns
	-1	99	-1	0.1503	ns		-1	101	1	0.7202	ns
	0	102	2	0.1353	ns		0	100	0	0.7240	ns
	1	107	7	0.0177	ns		1	132	32	0.0000	***
	2	112	12	0.0014	ns		2	118	18	0.0000	***
	3	116	16	0.0004	***		3	113	13	0.0010	**
	4	123	23	0.0000	***		4	112	12	0.0002	***
	5	128	28	0.0000	***		5	116	16	0.0001	***
	6	128	28	0.0000	***		6	111	11	0.0006	***
	7	129	29	0.0000	***		7	115	15	0.0001	***
	8	125	25	0.0000	***		8	115	15	0.0000	***
	9	122	22	0.0000	***		9	109	9	0.0029	**
	10	123	23	0.0000	***		10	107	7	0.0218	ns

3.3.4c: Spectral edge frequency differences from baseline for each minute before and after treatment.

Lignocaine						O Ring					
F95						F95					
	time	mean	difference	p	summary		time	mean	difference	p	summary
	-4	98.3	-1.7	0.0703	ns		-4	101	1	0.1203	ns
	-3	99.2	-0.8	0.3089	ns		-3	101	1	0.1826	ns
	-2	99.6	-0.4	0.4690	ns		-2	101	1	0.0987	ns
	-1	100.0	0.0	0.3220	ns		-1	100	0	0.6669	ns
	0	100.0	0.0	0.3154	ns		0	100	0	0.6699	ns
	1	99.3	-0.7	0.3593	ns		1	103	3	0.0008	***
	2	99.1	-0.9	0.3166	ns		2	104	4	0.0001	***
	3	96.6	-3.4	0.0071	**		3	103	3	0.0008	***
	4	94.1	-5.9	0.0000	***		4	104	4	0.0000	***
	5	95.1	-4.9	0.0003	***		5	104	4	0.0000	***
	6	95.8	-4.2	0.0007	***		6	104	4	0.0000	***
	7	96.5	-3.5	0.0018	**		7	104	4	0.0000	***
	8	96.0	-4.0	0.0004	***		8	104	4	0.0000	***
	9	95.2	-4.8	0.0001	***		9	102	2	0.0255	ns
	10	96.5	-3.5	0.0025	**		10	101	1	0.3025	ns

Table 3.3.5 Summary of t-tests performed to compare post treatment measurements with pre-treatment baseline measurements of Total Power, Median Frequency and 95% spectral edge frequency from electroencephalogram recording from rising one-year-old male deer that were treated with either a lignocaine ring block at the base of the antler or a NaturO™ ring. Tests were done on averages for each 15 second time period from two minutes prior to treatment until five minutes post treatment. Lignocaine and O Ring results are shown side by side for each of the variables measured.

** p<0.01

*** p<0.001

ns: Not significant

	Lignocaine					NaturO				
	Time	mean	diff	p	summary	Time	mean	diff	p	summary
TP	-120	101	1	0.601	ns	-120	102	2	0.465	ns
	-105	101	1	0.378	ns	-105	100	0	0.953	ns
	-90	103	3	0.023	ns	-90	99	-1	0.391	ns
	-75	101	1	0.549	ns	-75	100	0	0.937	ns
	-60	101	1	0.762	ns	-60	103	3	0.176	ns
	-45	97	-3	0.015	ns	-45	101	1	0.685	ns
	-30	99	-1	0.679	ns	-30	99	-1	0.531	ns
	-15	98	-2	0.191	ns	-15	97	-3	0.059	ns
	0	103	3	0.057	ns	0	97	-3	0.082	ns
	15	99	-1	0.401	ns	15	106	6	0.003	**
	30	98	-2	0.257	ns	30	112	12	0.003	**
	45	100	0	0.931	ns	45	102	2	0.397	ns
	60	106	6	0.001	**	60	100	0	0.997	ns
	75	103	3	0.043	ns	75	89	-11	0.000	***
	90	104	4	0.004	**	90	96	-4	0.098	ns
	105	104	4	0.015	ns	105	93	-7	0.002	**
	120	108	8	0.000	***	120	95	-5	0.010	ns
	135	108	8	0.000	***	135	92	-8	0.000	***
	150	107	7	0.000	***	150	96	-4	0.019	ns
	165	112	12	0.000	***	165	97	-3	0.095	ns
	180	107	7	0.000	***	180	98	-2	0.217	ns
	195	111	11	0.000	***	195	96	-4	0.020	ns
	210	109	9	0.000	***	210	98	-2	0.222	ns
	225	112	12	0.000	***	225	102	2	0.342	ns
	240	110	10	0.000	***	240	97	-3	0.089	ns
	255	114	14	0.000	***	255	97	-3	0.079	ns
	270	111	11	0.000	***	270	98	-2	0.342	ns
	285	113	13	0.000	***	285	95	-5	0.011	ns
	300	111	11	0.000	***	300	99	-1	0.749	ns

Table 3.3.5 continued

	<i>Lignocaine</i>					<i>NaturO</i>				
	Time	mean	diff	p	summary	Time	mean	diff	p	summary
<i>F50</i>	-120	105	5	0.1221	ns	-120	110	10	0.002	*
	-105	96	-4	0.1439	ns	-105	104	4	0.208	ns
	-90	98	-2	0.4253	ns	-90	98	-2	0.518	ns
	-75	101	1	0.7434	ns	-75	99	-1	0.552	ns
	-60	99	-1	0.7980	ns	-60	104	4	0.299	ns
	-45	107	7	0.0443	ns	-45	104	4	0.217	ns
	-30	109	9	0.0074	*	-30	100	0	0.959	ns
	-15	98	-2	0.3908	ns	-15	100	0	0.918	ns
	0	95	-5	0.0672	ns	0	94	-6	0.042	ns
	15	102	2	0.5677	ns	15	116	16	0.001	**
	30	101	1	0.7371	ns	30	124	24	0.000	***
	45	104	4	0.2973	ns	45	136	36	0.000	***
	60	122	22	0.0000	***	60	151	51	0.000	***
	75	110	10	0.0077	**	75	128	28	0.000	***
	90	113	13	0.0007	***	90	119	19	0.000	***
	105	109	9	0.0147	ns	105	109	9	0.013	ns
	120	118	18	0.0000	***	120	116	16	0.000	***
	135	123	23	0.0000	***	135	105	5	0.167	ns
	150	111	11	0.0040	**	150	112	12	0.000	***
	165	115	15	0.0000	***	165	116	16	0.000	***
	180	117	17	0.0000	***	180	116	16	0.000	***
	195	122	22	0.0000	***	195	115	15	0.000	***
	210	122	22	0.0000	***	210	107	7	0.034	ns
	225	125	25	0.0000	***	225	111	11	0.001	**
	240	122	22	0.0000	***	240	114	14	0.000	***
	255	126	26	0.0000	***	255	115	15	0.000	***
	270	127	27	0.0000	***	270	121	21	0.000	***
	285	133	33	0.0000	***	285	114	14	0.000	***
	300	125	25	0.0000	***	300	115	15	0.000	***

	<i>Lignocaine</i>					<i>NaturO</i>				
	Time	mean	diff	p	summary	Time	mean	diff	p	summary
<i>F95</i>	-120	99	-1	0.152	ns	-120	101	1	0.211	ns
	-105	100	0	0.453	ns	-105	101	1	0.107	ns
	-90	99	-1	0.060	ns	-90	100	0	0.574	ns
	-75	100	0	0.607	ns	-75	100	0	0.963	ns
	-60	100	0	0.850	ns	-60	100	0	0.956	ns
	-45	101	1	0.128	ns	-45	100	0	0.516	ns
	-30	100	0	0.738	ns	-30	100	0	0.724	ns
	-15	101	1	0.066	ns	-15	100	0	0.591	ns
	0	99	-1	0.310	ns	0	100	0	0.891	ns
	15	99	-1	0.273	ns	15	99	-1	0.343	ns
	30	101	1	0.328	ns	30	99	-1	0.530	ns
	45	99	-1	0.139	ns	45	103	3	0.001	**
	60	99	-1	0.023	ns	60	104	4	0.000	***
	75	99	-1	0.201	ns	75	106	6	0.000	***
	90	100	0	0.936	ns	90	105	5	0.000	***
	105	100	0	0.922	ns	105	104	4	0.000	***
	120	99	-1	0.032	ns	120	103	3	0.000	***
	135	98	-2	0.004	**	135	103	3	0.000	***
	150	99	-1	0.037	ns	150	102	2	0.004	**
	165	97	-3	0.000	***	165	103	3	0.001	**
	180	96	-4	0.000	***	180	103	3	0.000	***
	195	95	-5	0.000	***	195	103	3	0.000	***
	210	95	-5	0.000	***	210	104	4	0.000	***
	225	94	-6	0.000	***	225	102	2	0.004	**
	240	94	-6	0.000	***	240	104	4	0.000	***
	255	93	-7	0.000	***	255	105	5	0.000	***
	270	95	-5	0.000	***	270	105	5	0.000	***
	285	95	-5	0.000	***	285	105	5	0.000	***
	300	95	-5	0.000	***	300	103	3	0.000	***

Lignocaine vs NaturO

TP was higher in the LA group than the *NaturO* during the second minute after treatment ($p < 0.001$) and continued to be significantly higher until the end of recording. This difference was mainly attributable to the increase in the LA group, and to a lesser degree, a decrease in the *NaturO* group.

Median Frequency (F50)

Lignocaine vs Baseline

Lignocaine caused an gradual increase in F50 during the first five minutes. This was significantly greater than baseline by three minutes after application of the local anaesthetic and remained elevated until the end of recording.

NaturO vs Baseline

NaturOTM rings caused an increase in F50 that had a different pattern from that seen in the LA group. The maximum change was seen during the first minute when F50 was 38% greater than baseline ($p < 0.001$). This value then declined over time, but remained significantly elevated until the ninth minute after treatment.

Lignocaine vs NaturO

Both the Lignocaine and the *NaturO* groups demonstrated significant increases in F50 after treatment. During the first minute, F50 was significantly higher in the *NaturO* group than the Lignocaine group. This was the only time that a significant difference in one-minute means was recorded between treatments.

Spectral Edge Frequency (F95)

Lignocaine vs Baseline

Lignocaine caused a decrease in mean F95 that started about two minutes ($p < 0.01$) after treatment and then declined steadily until about five minutes after treatment ($p < 0.001$) after which it remained below baseline until the end of recording.

NaturO vs Baseline

NaturOTM rings caused a significant ($p < 0.001$) increase in F95 compared to baseline. This increase was first evident during the first minute after treatment and remained elevated until eight minutes after treatment. Graphically the mean spectral edge frequency increased during the first minute, then declined towards baseline, but remained above 100% for the remainder of the recording time.

Lignocaine vs NaturO

Spectral edge frequency was significantly lower in the LA group than the *NaturO* group from the second to the ninth minute after treatment. This was attributable to a rise in mean F95 in the *NaturO* group and a decrease in mean F95 in the LA group.

Individual animal results

Figures 3.3.5 and 3.3.6 show mean (solid black line) and individual animal profiles of TP, F50 and F95 for the lignocaine (Fig 3.3.5) and *NaturO*TM ring (Fig 3.3.6) groups. Figures 3.3.7 and 3.3.8 show significant ($P < 0.01$) increases or decreases relative to the average baseline for individual animals in channels one and two for animals treated with lignocaine (3.3.7) or a *NaturO*TM ring (3.3.8). Significant increases are shown in pink and significant decreases are shown in blue.

In the LA group, deer 1, 3, 5 and 17 demonstrate a pattern of increased total power, increase F95 and decreased F50. Deer 7, 9, 13 and 19 show some changes with similar trends although these are less consistent and not across all three variables (TP, F50 and F95) and Deer 11 and 15 in this group show very few changes over time.

In the *NaturO* group, deer 2, 10, 12(one channel only), 18 and 20 (one channel only) show a pattern of significant increases in F50 and F95, along with decrease in TP during the first few minutes. There were differences in the timing of these changes after application of the *NaturO*TM ring between deer and between channels in some deer. Deer, 4, 6, 8, 16 and 20 (channel 2 only) do not appear to show any recognisable patterns of change following application of the *NaturO*TM rings. The remaining deer show some significant responses, but these are inconsistent and difficult to interpret.

Discussion

For a full discussion of the results and methods of this experiment please refer to the general discussion at the end of this chapter

Conclusions

*NaturO*TM rings cause changes in the EEG from baseline that are consistent with a noxious somatosensory stimulus. These effects may be seen in some deer and not others.

Lignocaine causes changes in the EEG spectrum that have not been described previously. This was characterised by increased total power, increased median frequency and decreased spectral edge frequency. This suggests that there is increased activity in the frequency range somewhere between median and spectral edge frequency. This pattern is consistent with deepening anaesthesia rather than a noxious stimulus and is possibly due to central effects of lignocaine on brain activity.

There are significant differences between *NaturO*TM rings and Lignocaine on the EEG spectrum.

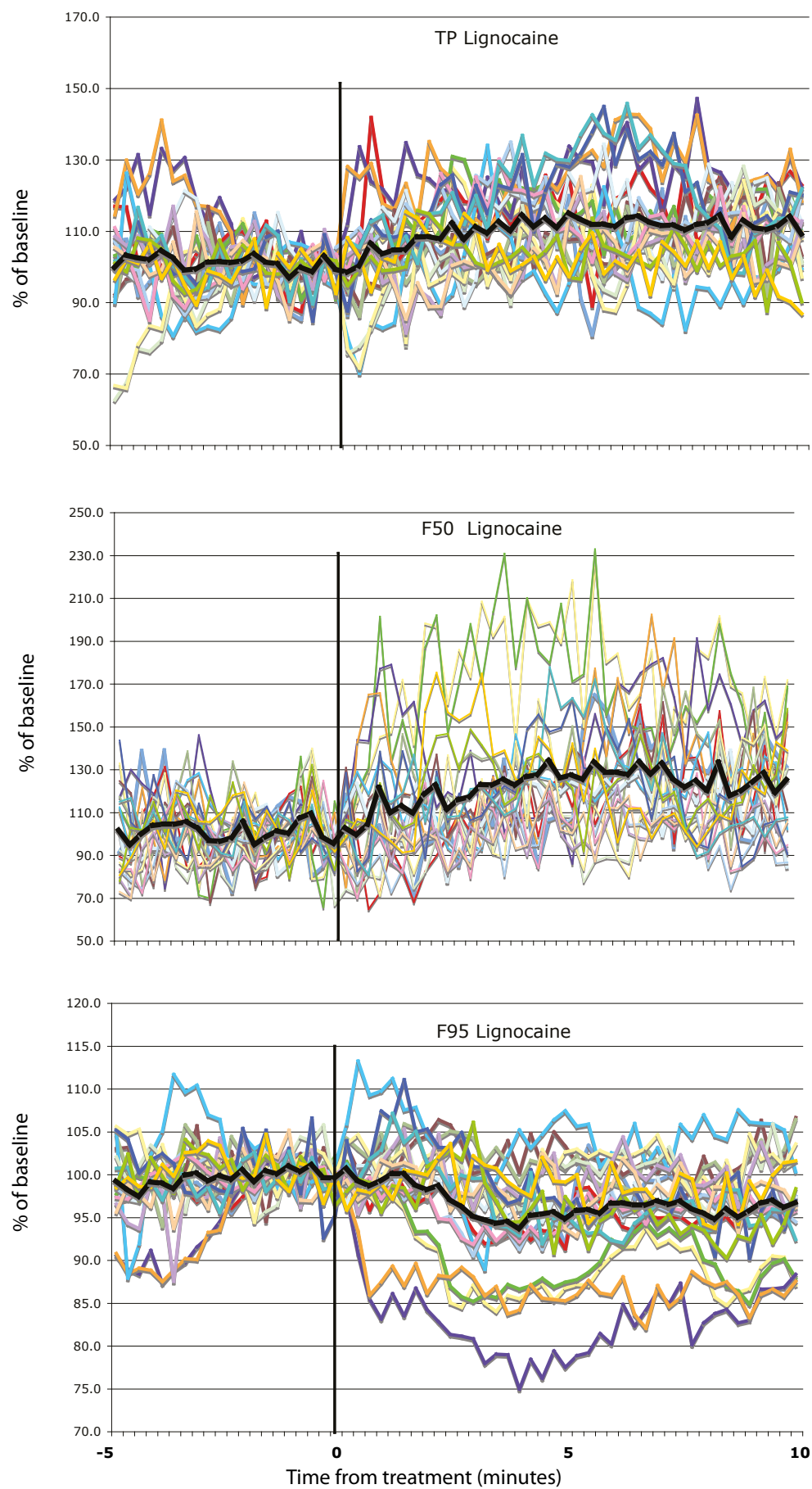


Figure 3.3.5 Individual animal 15-second means as a percent of baseline plotted on the same graph panel along with overall group means (thick black line) for Total power (a), F50 (b) and F95 (c) from five minutes before treatment until 10 minutes after treatment with lignocaine

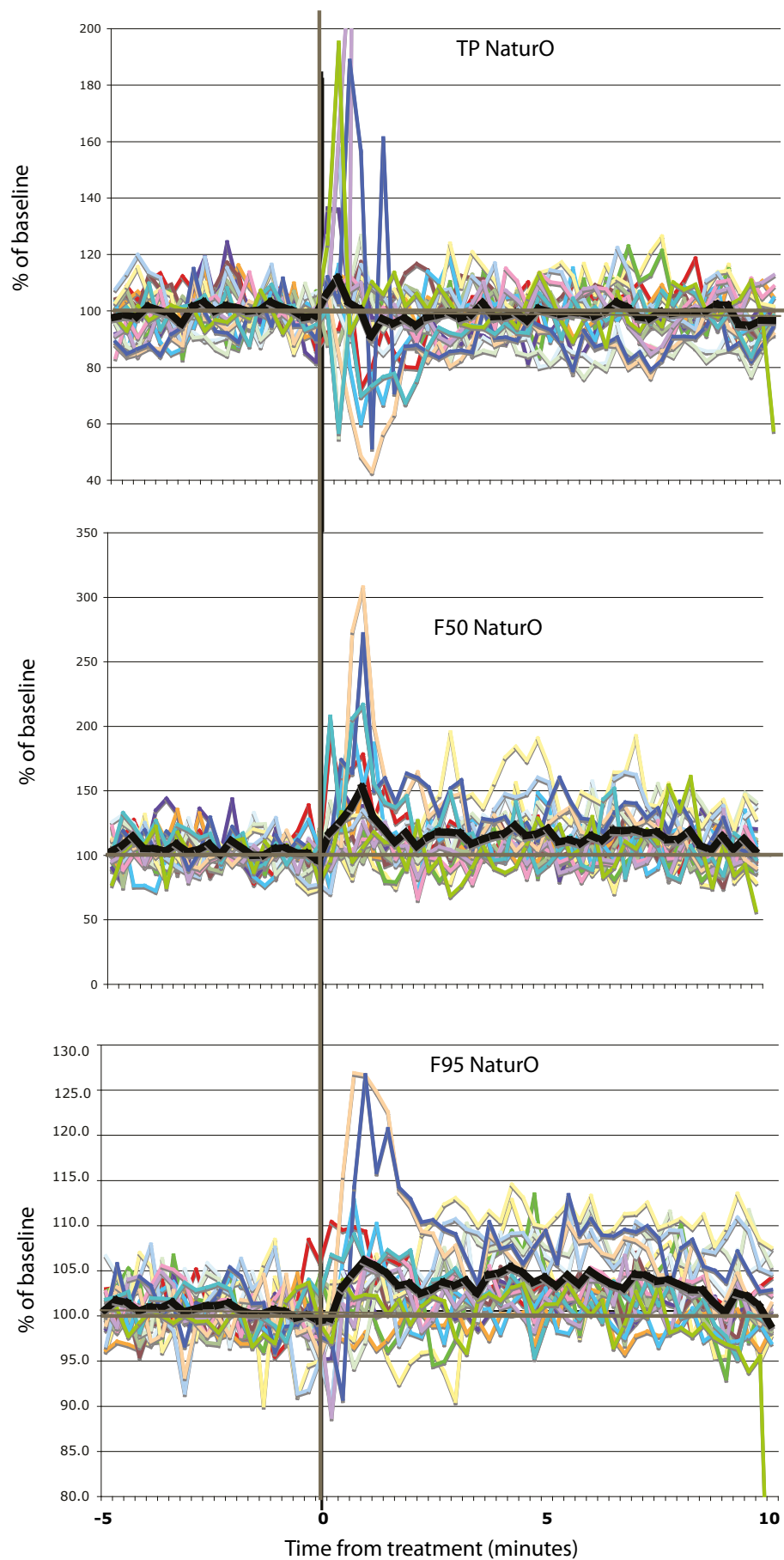


Figure 3.3.6 Individual animal 15-second means as a percent of baseline plotted on the same graph panel along with overall group means (thick black line) for Total power (a), F50 (b) and F95 (c) from five minutes before treatment until 10 minutes after treatment with NaturO™ rings

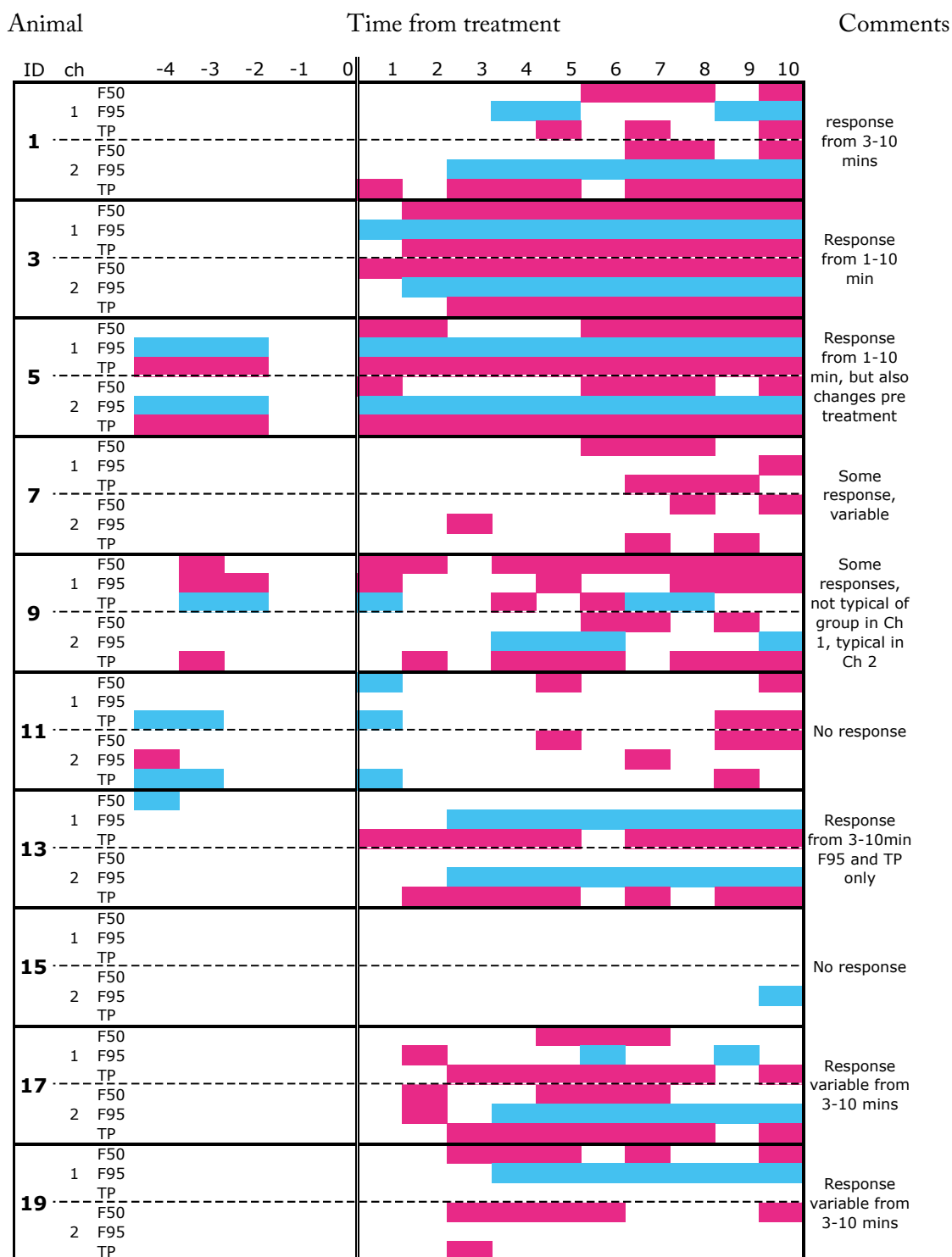


Figure 3.3.7 Schematic diagram showing significant changes from pre-treatment baseline values for F50, F95 and TP for each minute from 5 minutes before treatment to ten minutes after treatment with a lignocaine ring block around the base of the pedicle of yearling stags.

Each number in the left hand column represents an individual animal and the second column represents the two channels of EEG. Significant ($p < 0.01$) increases are shown in pink and significant decreases in blue. Where there was no significant change from baseline the cells are white.

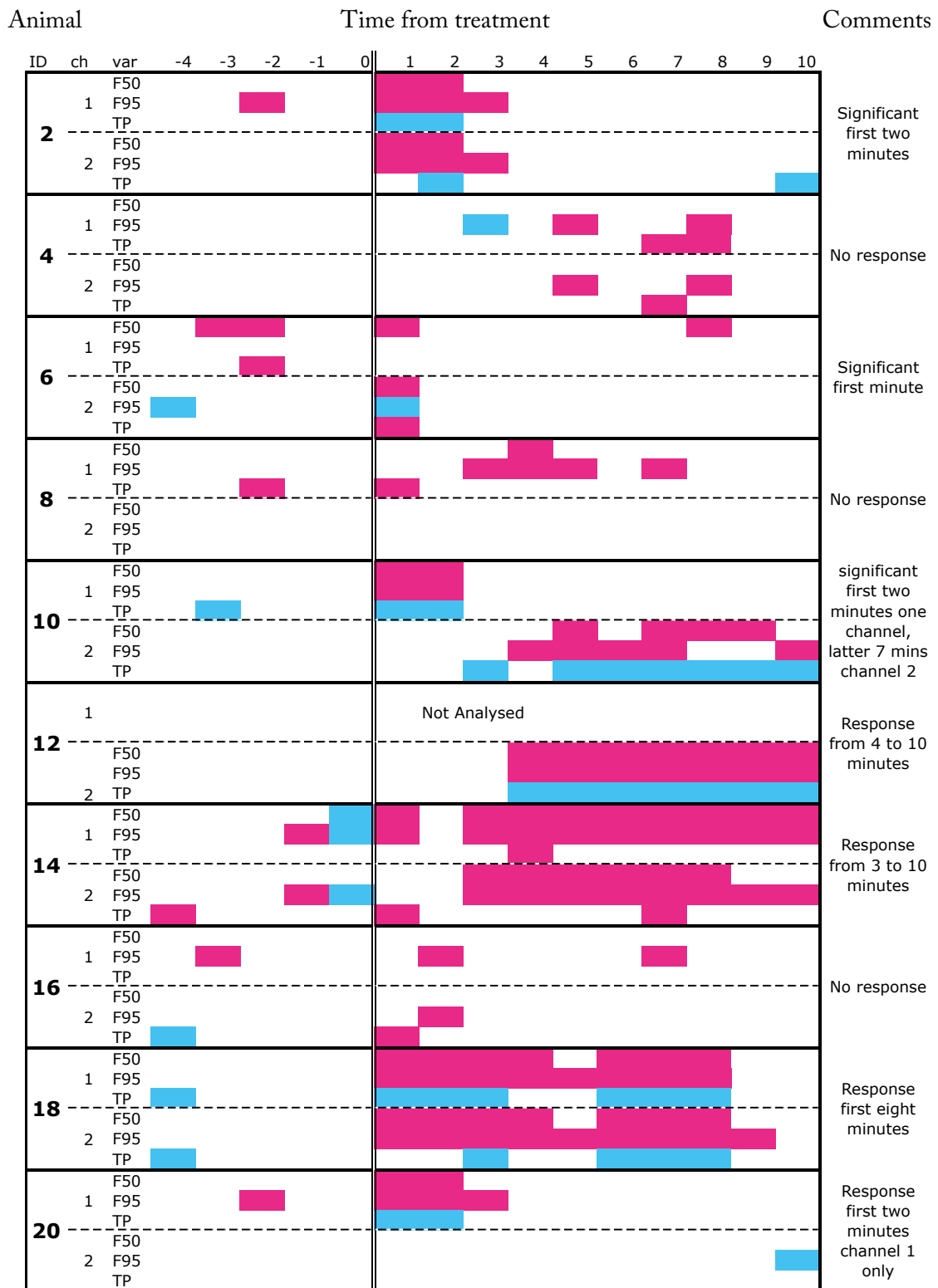


Figure 3.3.8 Schematic diagram showing significant changes from pre-treatment baseline values for F50, F95 and TP for each minute from 5 minutes before treatment to ten minutes after treatment with a NaturO™ ring around the base of the pedicle of yearling stags.

Each number in the left hand column represents an individual animal and the second column represents the two channels of EEG. Significant ($p < 0.01$) increases are shown in pink and significant decreases in blue. Where there was no significant change from baseline the cells are white.

3.4 Effects of the application of lignocaine ring blocks and/or NaturO™ rings on the behaviour of one-year-stags

Introduction

This study aimed to determine whether there are significant differences in the behaviour of yearling stags that have had NaturO™ rings applied, local anaesthetic applied or no treatment applied to one or both antlers.

Materials and Methods

Animals

One hundred rising-one-year-old male red deer were used. These were made up of animals born and reared on the Massey University Deer Unit and a cohort of animals brought in from two different commercial farms and managed on the Massey University Deer Unit. The brought-in

deer arrived on 17 August 2007 and experimental observations were made from October 2007 to January 2008.

Table 3.4.1 Treatment groups and descriptions for observations of the effects of NaturO™ rings on the behaviour of yearling stags

Group	Treatment	Description
1	Control	No treatment (Velcro)
2	NaturO both	NaturO™ ring on both antlers + Velcro band both
3.	NaturO left	NaturO™ ring on left pedicle, Velcro on both
4	NaturO right	NaturO™ ring on right pedicle, Velcro on both
5	Lignocaine both	Lignocaine both pedicles, Velcro on both
6	Lignocaine left	Lignocaine on left pedicle, Velcro on both
7	Lignocaine right	Lignocaine on right pedicle, Velcro on both
8	Lignocaine and NaturO, both	Lignocaine on both pedicles followed by NaturO™ ring on both, Velcro on both
9	Lignocaine left, NaturO right	Lignocaine on left pedicle, NaturO™ ring on right, Velcro on both
10	NaturO left, Lignocaine right	NaturO™ ring on left pedicle, lignocaine on right, Velcro on both.

Treatments

All deer were observed on two occasions being pre-treatment (“pre”) and treatment (“post”). For the pretreatment observations, all deer had a Velcro band placed around each pedicle. In the treatment observations, all deer had Velcro bands placed over the treatment site on both antler pedicles so that the person analysing the video tapes would not know the treatment or whether the recording was from a “pre” or “post” treatment observation.

Deer were randomly assigned to one of the treatments (10 per treatment) shown in Table 3.4.1

Lignocaine: Local anaesthetic (Lignocaine hydrochloride 2%, “Nopaine”, Phoenix Pharm.) was placed around base of the pedicle in a continuous ring, at a dose of 1mL per centimetre pedicle circumference.

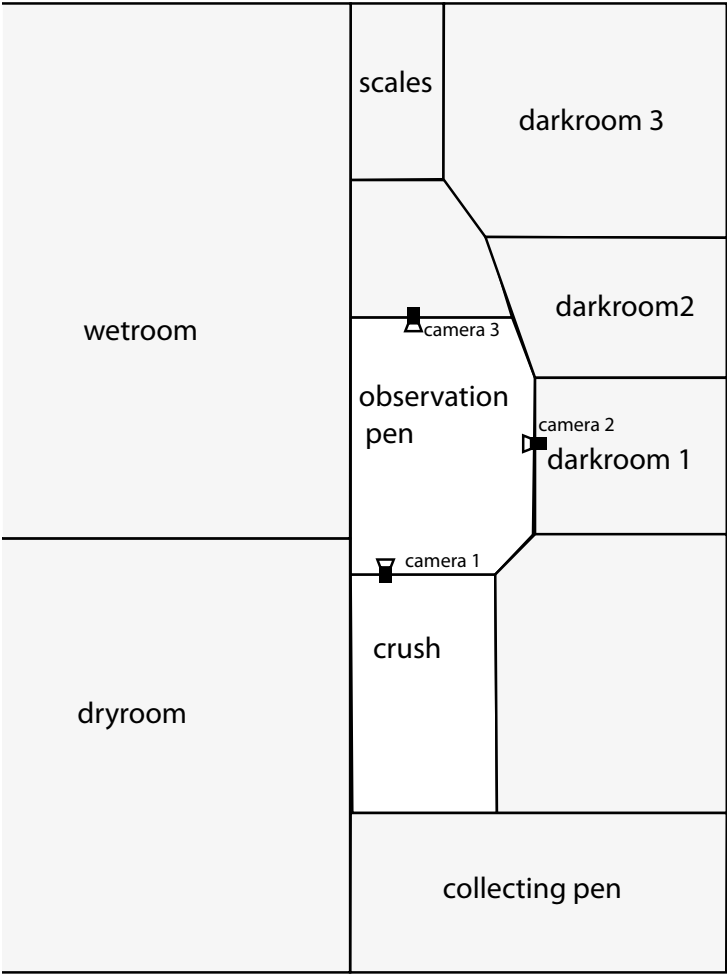
NaturO: NaturO™ rings were applied using the cable-tied method. The cable tie was cut short using heavy duty scissors so that the Velcro would hide the treatment.

Procedure

Habituation

Because of the variable results described earlier, deer were habituated for six weeks before the start of video observations. This included bringing the animals through the yards twice a week and weighing once a week. During the 6th week the deer were held in the observation pen for 5 to 10 minutes at each handling occasion.

Video recording



Three Sony handycam video cameras were set up above the observation pen. The arrangement of the pens and cameras is shown in figure 3.4.1 and figure 3.4.2 shows the deer in the observation pen with the identifying colours and velcro bands on. One deer has a velcro band removed to show positioning of the NaturO™ ring. The observation pen was located immediately following the crush. The cameras were placed to cover all angles of the pen and thus record observations of all deer at all times. Video data were recorded onto miniDV cassettes and stored for data retrieval and analysis at a later date. An incandescent work-light was placed above the crush to allow better visualisation of the identifying colours on the deer to mark them for behavioural analysis from the videos.

Figure 3.4.1 Layout of the crush and observation pens for video recording the behaviour of yearling stags after the application of NaturO™ rings



Figure 3.4.2 Deer in the observation pen for videoing of behaviour during one hour after placement of local anaesthetic or NaturO™ rings. Each deer is marked with a different coloured collar and rattle. The velcro band has been removed from the antler of one deer to show placement of the NaturO™ ring.

Deer were brought into the crush in a group of 5 randomly assigned to treatment groups and marked with a colour, either blue, green, yellow, orange or pink. The colour included spray rattle on the back of the deer and a plastic collar of the same colour (Figure 3.4.2).

Deer were restrained in a hydraulic crush two or three at a time and velcro bands were applied (pretreatment) or the treatment was applied and a velcro band placed over the site of the treatment.

Deer were then released into the observation pen in a group of five and left undisturbed for one hour. No other activities took place in or around the deer shed during the times of video recording.

After one hour of video recording the collars, velcro bands

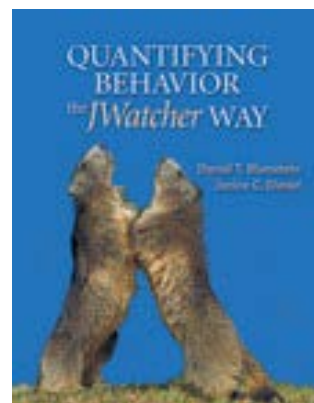


Figure 3.4.3 Book to accompany JWatcher software

and NaturO™ rings (if any) were removed and the deer were released back to the paddock. Velvet was not removed at this time.

The same five deer were in the same group for pre and post- treatment observations. Pre-treatment observations were always conducted before post-treatment.

JWatcher software (Blumstein and Evans) was used to capture data from the videos following instructions in a book written by the same authors (Figure 3.4.3)). I determined how to use the software and which behaviours would be recorded. A technician was then trained to score behaviours on a full-time basis. This was to ensure consistency in scoring between animals without distractions or bias as discussed later.

Initially scoring was done using JWatcher-video and quick-time movie files. It was found that an experienced analyst could live score from the original video tapes and use the pause, slow motion and editing functions to correct errors in data entry, reducing the need to convert videos from one format to another. Every video was watched twice by the same person.

An example of the *JWatcher* user interface is shown in Figure 3.4.4

Behavioural ethograms and use of JWatcher

JWatcher-video version 1.0 was installed onto a Macbook Pro and iMac using Java version 12.2.0. under a Mac 10.5.8 operating system. Quicktime version 7.6.2 was installed to run videos.

Following the instructions in the book, a Global Definition file (*.gdf) was created that included 32 different behaviours. These are shown in the column headed “Global” in Table 3.4.2. These behaviours were determined by watching several randomly selected sections of video and recording every behaviour observed in that section. Further behaviours were added to the Global Definition file during data collection as the software allows new behaviours to be added if they are discovered after data collection has started. Each behaviour was represented by a letter code as indicated in the column on the left of Table 3.4.2. No modifiers were included in the Global Definition file.

From the Global Definition ethogram, a Focal Master file (*.fmf) was created for scoring the behaviours from the video tapes. In this experiment all of the behaviours in the Global definition file were included in the Focal Master file.

In the Focal Master file, the time was not set as not all deer entered the pen at the same time or at the start of the video recording.

Individual deer were then behaviour scored for one hour using the “Data Capture” function. Only the colour of the deer and the number of the video tape was known to the scorer and not treatment group or pre or post treatment observation. These files were saved as *.dat files and edited by rewatching the videos and using the “Edit Data” function.

After all 200 data files were generated, the data files were assessed briefly and several “Focal Analysis” files (*.faf) were created for performing different analyses. The analyses were run using

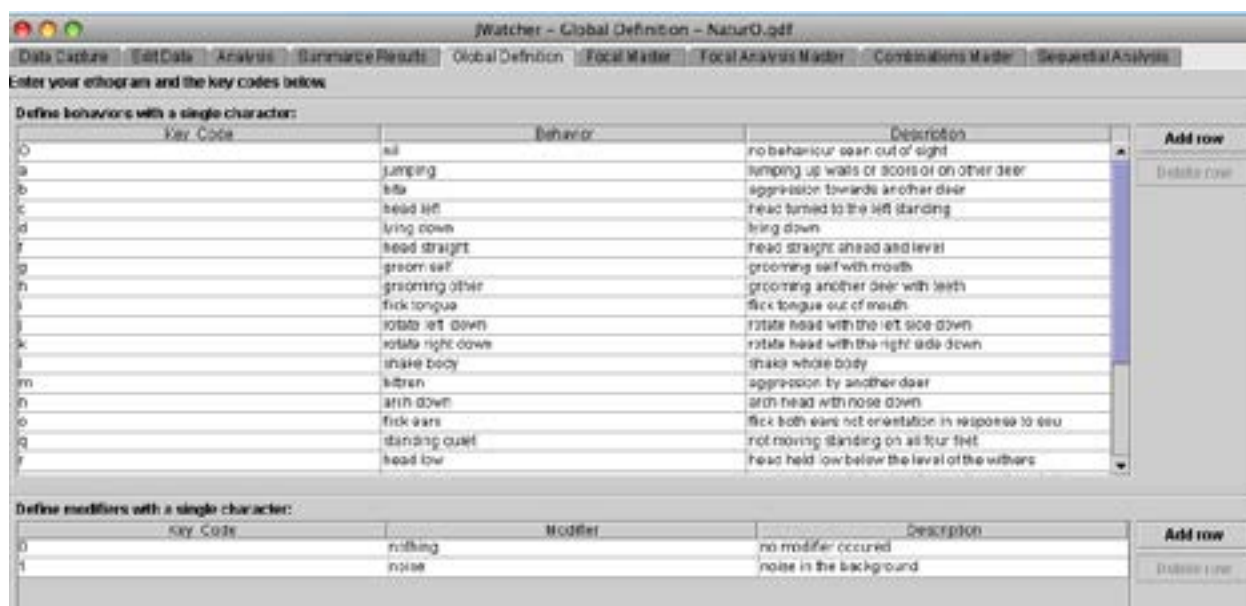


Figure 3.4.4 Interface for the JWatcher behavioural data collection software showing the global definition for the ethogram of behaviours and descriptions.

“Analyse Results” and generated individual results files (*.res) for each animal-observation period in comma delimited text file format.

Results files were then summarised into a comma delimited text file using the JWatcher “Summarize Results” function. The final comma delimited text file was then copy and pasted into an excel spreadsheet and converted to table format for further statistical analysis. After more detailed analysis further behaviours were removed from the final statistical analysis and graphs.

“Combination Master” and “Sequence Analysis” were not used in this study.

Data Analysis

Data analyses were performed on the output data from JWatcher using Microsoft excel and Prism 4 for Mackintosh software.

Grouping of data by treatment

Initial inspection of the data indicated similar behaviour patterns in deer in treatment groups 2, 3 and 4 (all NaturO™ ring), and in groups 5,6,and 7 (all local anaesthetic).

For most of the analyses treatment groups were combined as follows:

- Groups 1 and 8 were combined as “Control”
- Groups 2, 3 and 4 were combined as “NaturO”
- Groups 5, 6 and 7 were combined as “Lignocaine”
- Groups 9 and 10 were combined as “Both”

Table 3.4.2 Behaviours included in the Global Definition, Focal Master, Analysis and Statistical Ethograms

State behaviours					
Code	Description	Global	Focal	Analysis	Statistics
Whole body position					
x	Standing still	✓	✓	✓	✓
w	Walking	✓	✓	✓	✓
t	Pacing	✓	✓	✓	✓
q	Walk backwards	✓	✓	✓	✓
m	Circling	✓	✓	✓	✓
i	Lying down	✓	✓	✓	✓
r	Running	✓	✓	✓	✗
Head position (Analysed as both state and event behaviours)					
a	Head straight	✓	✓	✓	✓
g	Head left	✓	✓	✓	✓
f	Head right	✓	✓	✓	✓
d	Head low	✓	✓	✓	✓
s	Head high	✓	✓	✓	✓
Event Behaviours					
Non-specific events					
G	Look up	✓	✓	✓	✗
h	Nose to ground	✓	✓	✓	✓
A	Lick the walls	✓	✓	✓	✓
o	Flick tongue	✓	✓	✓	✓
p	Flick ears	✓	✓	✓	✓
J	Fall down	✓	✓	✗	✗
S	Stand up	✓	✓	✗	✗
e	Jumping	✓	✓	✗	✗
b	Bite	✓	✓	✗	✗
n	Bitten	✓	✓	✗	✗
k	Groom self	✓	✓	✓	✗
l	Groom other	✓	✓	✓	✗
u	Scratch	✓	✓	✓	✗
z	Shake body	✓	✓	✓	✗
Specific head-related events					
c	Rotate head left down	✓	✓	✓	✓
v	Rotate head right down	✓	✓	✓	✓
F	Turn head in 360 circles	✓	✓	✓	✓
H	Arch head down	✓	✓	✓	✓
j	Arch head up	✓	✓	✓	✓
D	Press head	✓	✓	✓	✓
y	Out of sight	✓	✓	✓	✗

State and Event Behaviours

State behaviours were analysed as proportion of time spent in each mutually exclusive state. These were divided into whole body state positions and head state positions. Event behaviours were analysed as the number of times the behaviour occurred in a given time period. Events were not mutually exclusive.

The behaviours studied are listed in Table 3.4.2. For data analysis and presentation the behaviours were grouped according to the following categories.

Whole Body Position State

Mutually exclusive states analysed as proportion of time in each state including: “Standing”, “Walking”, “Walking backwards”, “Pacing”, “Circling”, “Lying down”.

Head Position State

Mutually exclusive states analysed as proportion of time in each state including: “Head Straight”, “Head Left”, “Head Right”, “Head low” and “Head High”.

Head Position Event

Independent events analysed as frequency (number per specified time period). Including “Head Straight”, “Head Left”, “Head Right”, “Head low” and “Head High”.

Non-specific Event

Independent events analysed as frequency (number per specified time period) Including: “Look up”, “Nose to ground”, “Lick the walls”, “Flick tongue”, “Flick ears”, “Fall down”, “Stand up”, “Jump” “Bite”, “Bitten”, “Groom self”, “Groom other”, “Scratch”, “Shake body”.

Specific Head Related Event

Independent events analysed as frequency (number per specified time period) Including: “Rotate left”, “Rotate right”, “Turn head 360°”, “Arch head down”, “Arch head up”, “Press head”.

Basic data distribution

The following basic statistics were recorded for each behaviour:

Number of animals positive

The number of animals that demonstrated the behaviour at least once during the “pre” and “post” treatment periods.

For behaviours that had a low number of animals that demonstrated the behaviour in all treatment groups during both the pre and post treatment periods, full data analysis was not performed.

Z-test for difference in proportion positive

A Z-test was performed to compare two sample proportions. If $p < 0.05$, the proportion positive during the “pre” and “post” periods were considered significantly different.

Data Distribution

Mean, Median (non parametric data) and range were determined for all behaviours for the “pre” and “post” treatment periods. Data distributions were presented as frequency histograms.

Test for normality

Data were tested using the D’Agostino & Pearson omnibus normality test. For data that had an approximately normal distribution, tests of equal means were performed in future analysis. Where data were not normally distributed, non-parametric analyses were performed. Many data were neither normally distributed nor parametric in nature, so to give an indication of the data spread, raw data points are shown where possible.

Correlation analyses

Data from the “pre” treatment recording was compared with that from the “post” treatment recording for individual animals to determine whether there was a correlation between the two recording periods. This was to investigate whether individual animals had a repeatable pattern of behaviour during penning for one hour. i.e. to determine whether an animal that demonstrated a certain behaviour at a high frequency on the first occasion would be likely to demonstrate the same behaviour with a high frequency on the second occasion. Pearson R^2 and values for significant deviation from zero slope were calculated for normally distributed data and Spearman R^2 for non-parametric data.

Within and between treatment group comparisons

Pre vs Post comparisons within treatment groups

T-tests were conducted to determine whether there were significant differences between the “pre” and “post” treatment recording periods within each treatment group. For non-parametric data Wilcoxin Sign ranked tests were performed with individual animals considered as paired data. Differences were considered significant when $p < 0.05$.

Comparisons of “pre” and “post” between treatment groups

Two way ANOVA and post-ANOVA bonferroni analyses were done to determine whether there were differences between treatment groups in the “pre” and “post” treatment periods and whether there was a difference between treatments in the “difference” between “pre” and “post”.

For non-parametric data, one way ANOVA with Dunn’s post test comparisons were performed.

Difference (Post-Pre)

For individual animals the difference between the “post” treatment recording and the “pre” treatment recording was determined. This is shown graphically as individual dot plots.

Proportion of animals that had double (increase) or halving (decrease), or no change.

For each behaviour individual animals were scored as “no change”, “increase” or “decrease” based on the following equation:

$$\frac{(N_{\text{post}} - N_{\text{pre}})}{(N_{\text{pre}} + N_{\text{post}})/2}$$

The denominator was taken as the average of pre and post values to allow for zero values. If both pre and post were zero the result was an error (/0) in which case it was recorded as “no change”.

If the “post” treatment recording was double or half that of the “pre” treatment recording, the result of the above equation was 0.67. With a negative sign indicating a “decrease” and a positive sign indicating an “increase”. Any animal that had a 100% or greater increase (double) in frequency was scored as “increase”, any animal that had a 50% or greater decrease (halve) was scored as “decrease” and those that changed by less than this amount in either direction were scored as “no change”.

For each treatment, the number and net proportion of animals that showed a “increase”, “decrease” or “no change” for each behaviour was determined by the following equation:

$$\text{Net \% change} = \frac{N \text{ "increase"} - N \text{ "decrease"}}{N \text{ total}}$$

Where N is the number of animals and N total is the number in the treatment group.

Results were considered significant when the overall change was greater than 35% for “Control” and “Both” (N=18 for both of these groups) and greater than 30% for “NaturO” and “Lignocaine” (N= 30 and 29 respectively). These cut-off values were based on the difference in proportion that would be considered significant at a 95% confidence level given the specified sample sizes

Time series analysis

For behaviours of interest, the frequency was determined for each of the 10 minute time periods after the start of recording to see if there was a pattern of change over time. Trends were analysed by visual assessment of the graphical display.

Results

Film 12 was not able to be analysed because of poor lighting. This included deer 1I (treatment 1), 7I (treatment 7), 8I (treatment 8), 9F (treatment 9) and 10H (treatment 10). Correspondingly, each of these treatments had 9 animals for analysis. The combined treatment groups had the following number of animals

Control: 18

NaturO: 30

Lignocaine: 29

Both: 18

A summary of the statistical analysis results is provided in Table 3.4.13 at the end of this results section and descriptions and graphical displays are detailed here.

Whole body state behaviours

Whole body state behaviours - Individual behaviour statistics

Standing

All animals demonstrated the behaviour “Standing” during both the “pre” and “post” treatment observation periods. The mean proportion of time spent “Standing” was 0.52 and 0.47 for the pre and post treatment recordings respectively. This difference was not statistically significant. This ranged from 0.03 to 0.92 for individual animals. The data were approximately normally distributed. Figure 3.4.5 (top graph) shows the distribution of data for “Standing” for all deer during the pre and post treatment periods.

Walking

During the “pre” and “post” treatment periods, 92 and 93 out of 95 animals demonstrated the behaviour “Walking” respectively. The mean proportion of time spent “Walking” was 0.06 and 0.08 for “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 0 to 0.45. The data were not normally distributed and were skewed to the right. Figure 3.4.5 (middle graph) shows the distribution of data for “Walking” for all deer during the “pre” and “post” treatment periods.

Walking Backwards

During the “pre” and “post” treatment periods, 63 and 65 out of 95 deer demonstrated the behaviour “Walk backwards”. The mean proportion of time spent walking backwards was 0.02 for both “pre” and “post” treatment recordings. The range was 0 to 0.24. The data were not normally distributed. Figure 3.4.5 (lower graph) shows the frequency distribution of “Walking backwards” for all deer during the “pre” and “post” treatment recordings.

Pacing

All deer demonstrated the behaviour “Pacing” during both the “pre” and “post” treatment observation periods. The mean proportion of time spent “Pacing” was 0.37 and 0.39 for “pre” and “post” treatment recordings respectively. The range was 0.002 to 0.77. The data were approximately normally distributed. Figure 3.4.6 (top graph) shows the frequency distribution for “Pacing” for all deer during the “pre” and “post” treatment recordings.

Lying Down

Eight deer demonstrated the behaviour “Lying down” during the “pre” treatment period and four during the “post” treatment period. This difference was not statistically significant. The mean proportion of time spent “Lying down” was 0.013 for both “pre” and “post” treatment recordings. The median time was 0 and the range was 0 to 0.44. Data were not normally distributed and were skewed to the right. There were insufficient data to make comparisons between treatments. Figure 3.4.6 (middle graph) shows the frequency distribution of “Lying down” for all deer during the “pre” and “post” treatment periods.

Circling

Five deer demonstrated “Circling” during the “pre” treatment period and 16 during the “post” treatment period. This difference was significant ($p=0.011$). The mean proportion of time spent “Circling” was 0.0003 and 0.0028 for “pre” and “post” treatment periods respectively. The median was 0 and the range was 0-0.06. Data for “Circling” were not normally distributed and skewed to the right. Figure 3.4.6 (lower graph) shows the frequency distribution of “Circling” for all deer during the “pre” and “post” treatment periods.

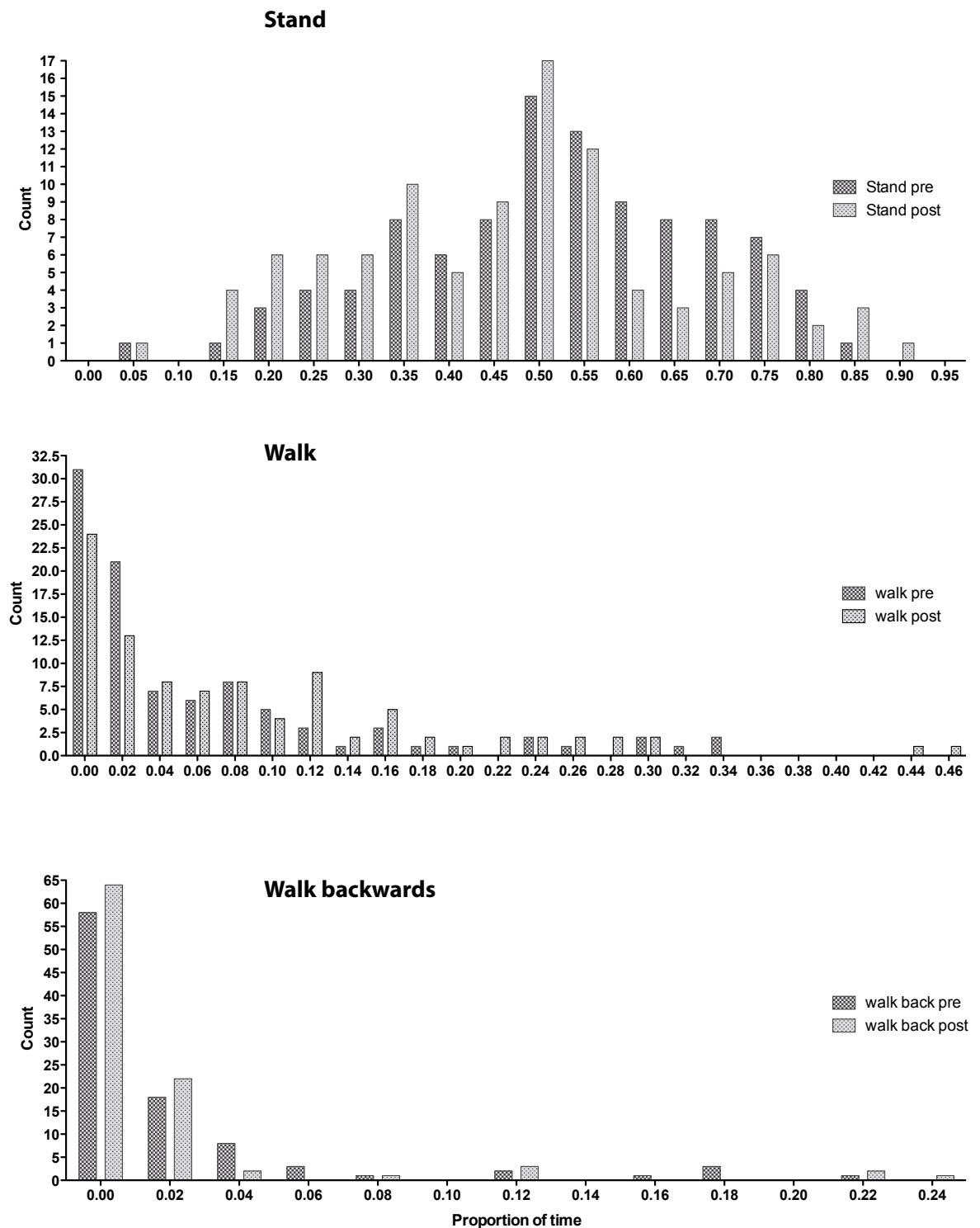


Figure 3.4.5 Histograms of the distribution of the proportion of time that yearling stags spent in the “Standing”, “Walking” and “Walking backwards” behaviour states during one hour of video observation in a pen either before (pre) or after (post) treatment with either NaturO™ rings, lignocaine, no treatment or a combination of these. “Pre” treatment values are shown in dark fill and “Post” treatment values in light fill.

Running

Nine deer demonstrated the behaviour “Running” during both the “pre” and “post” treatment periods. The mean proportion of time spent “Running” was 0.0003. “Running” was not analysed further.

Whole body state behaviours pre vs post correlations

Figure 3.4.7 shows the proportion of time spent in three different state behaviours (“Pacing”, “Walking” and “Walking backwards”) during the pre-treatment recording (x axis) and post-treatment recording (y axis). The time bin used for these correlation analyses was 60 minutes. States that included active movement by the animal were combined and called “Active” state. Figure 3.4.8 shows scatterplots of the proportion of time spent in either “Standing still” or “Active” state behaviour during the “pre” treatment recording (x axis) and during the “post” treatment recording (y axis). In these figures, each animal is colour coded according to treatment “Type” (“Control”, “NaturO”, “Lignocaine”, “Both”). Correlation analyses were done for all animals combined and for animals within the four different treatment groups. Table 3.4.3 shows Pearson R^2 or Spearman r correlation goodness of fit results and p-values to test if the correlation was significantly different from zero for whole body position states. Across all animals, there were significant correlations between the first and second recording period for all state behaviours except “Circling”. Within treatment groups there was variation in the significance of the correlation between “pre” and “post” for the difference whole body state behaviours. There were no behaviours that had significant correlations between “pre” and “post” treatment recordings across all treatment groups.

**Whole body state comparison of pre vs post within treatment
(paired t-test of means or Wilcoxin matched pairs signed ranked
test of medians)****Standing**

“Both” spent less time “Standing” during the “post” treatment period ($p=0.013$)

No other differences

Walking

“Both” spent more time “Walking: during the “post” treatment period ($p=0.0023$)

No other differences

Walking backwards

No differences

Pacing

No differences

Lying down

No difference

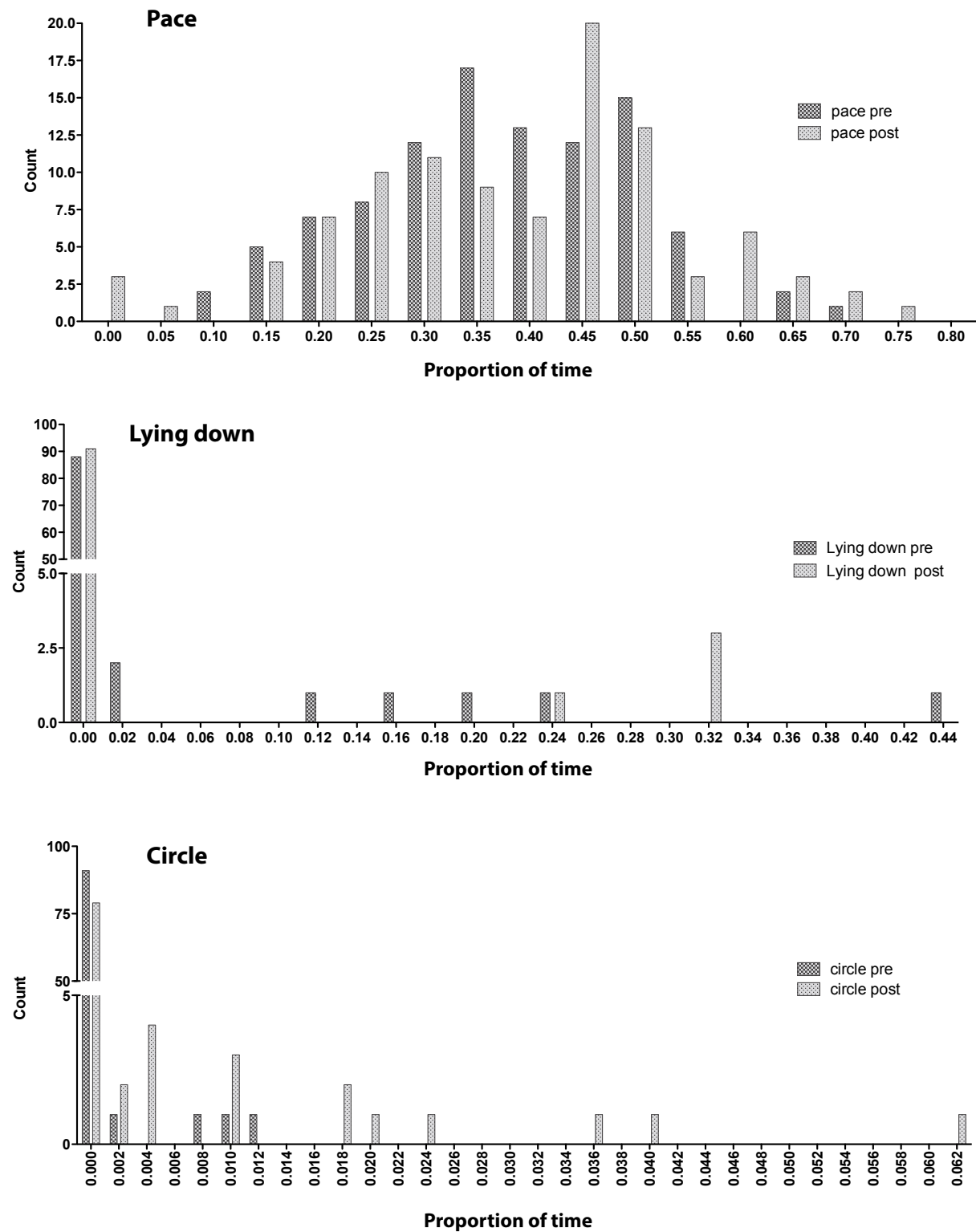


Figure 3.4.6 Histograms of the distribution of the proportion of time that yearling stags spent in the “Pacing”, “Lying down” and “Circling” behaviour states during one hour of video observation in a pen either before (pre) or after (post) treatment with either NaturO™ rings, lignocaine, no treatment or a combination of these. “Pre” treatment values are shown in dark fill and “Post” treatment values in light fill.

Circling

No difference

Between treatment comparisons. ANOVA and post ANOVA tests. Followed up with individual group t-tests or Mann Whitney

Figures 3.4.9 and 3.4.10 show individual data plots of the proportion of time that each animal spent in each whole body state behaviour during the “pre” treatment period and during the “post” treatment period for each treatment group. Two-way ANOVA analysis found no overall significant effect of treatment on the frequency of behaviours. When t-tests were run to compare treatment types the following significant differences were found.

Between treatment differences - “pre” treatment

“NaturO” “paced” less than “Control” ($p=0.035$)

“NaturO” “paced” less than “Lignocaine” ($p=0.0077$)

“NaturO” “paced” less than “Both” ($p=0.0024$)

Between treatment differences - “post” treatment

“NaturO” paced less than “Lignocaine” ($p=0.0032$)

“NaturO” paced less than “Both” ($p=0.0009$)

Difference (Post-Pre)

Figure 3.4.11 shows the difference in the proportion of time spent in each whole body state behaviour during the “post” treatment period minus the “pre” treatment period.

There were no differences between any treatment groups in the difference between “pre” and “post” treatment periods.

Proportion change for individual animals

The number of animals that doubled (“increased”) or halved (“decreased”) the proportion of time in each behaviour from the “pre” treatment recording to the “post” treatment recording was determined for each treatment group. Table 3.4.4 shows the number of animals that demonstrated an “increase”, “decrease” or “no change” for each whole body state behaviour for each treatment group.

There was a 67% net “increase” in the number of animals that changed the proportion of time “Walking” “post” treatment compared to “pre” treatment.

There were no other significant (net change $\geq 30\%$) net changes in the proportion of animals that “increased” or “decreased” the time spent in each whole body state.

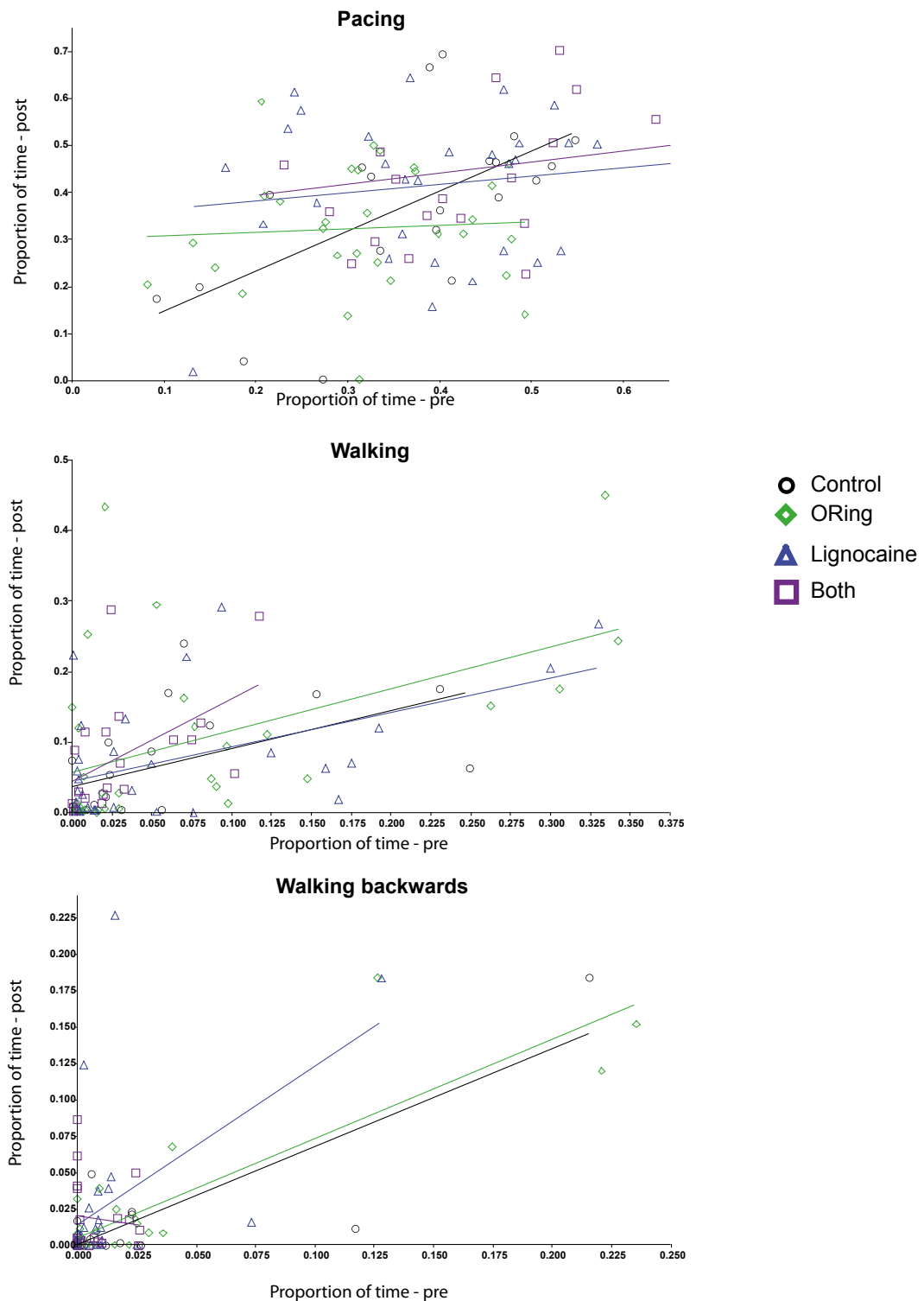


Figure 3.4.7 Scatterplots of the proportion of time that yearling stags spent in whole body state behaviours “Pacing”, “Walking” and “Walking backwards” during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with NaturO™ rings (NaturO, lignocaine ring blocks(Lignocaine) or no treatment (Control) or an NaturO on one antler and Lignocaine on the other antler (Both). Correlation analysis lines for each of the treatment groups are shown.

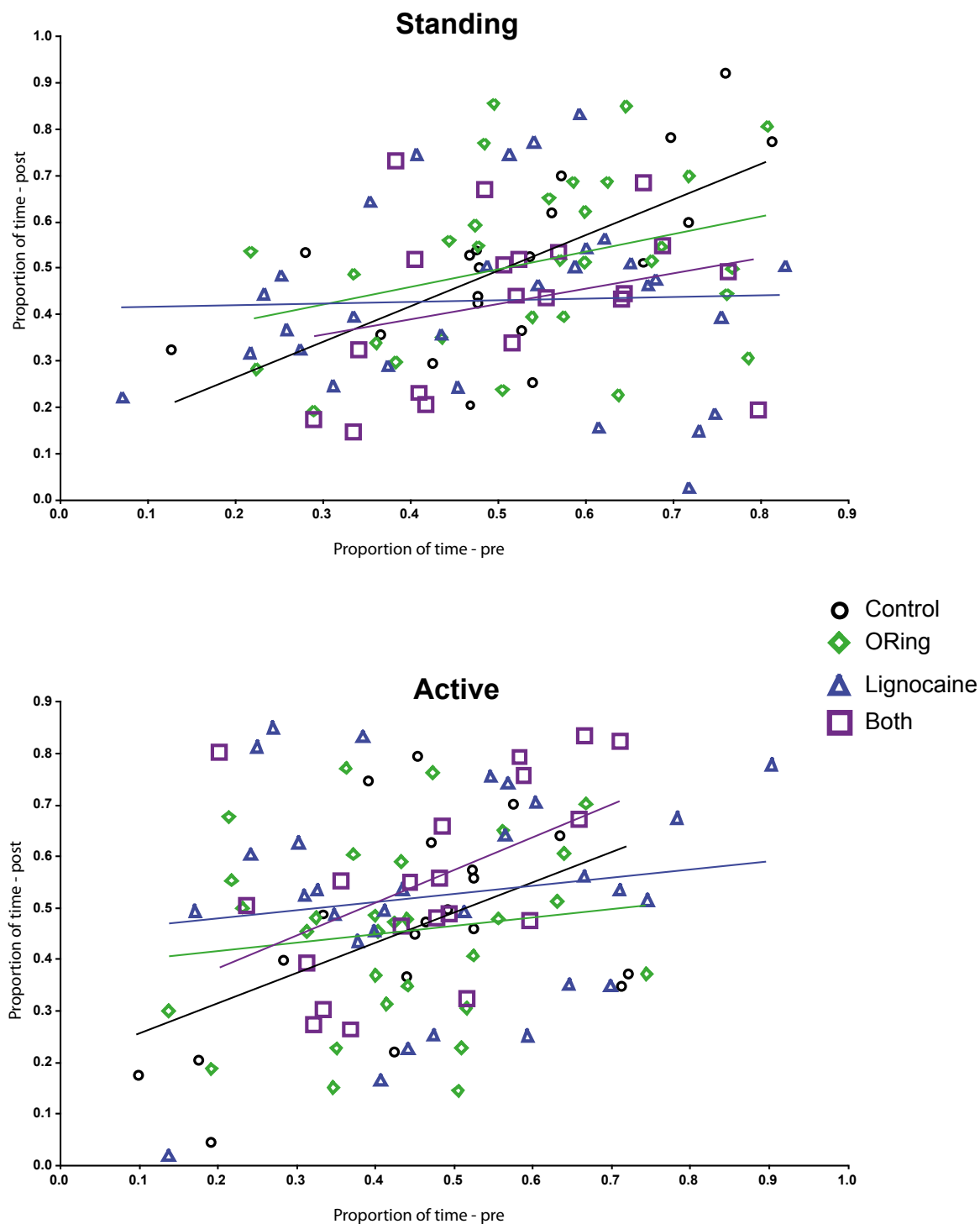


Figure 3.4.8 Scatterplots of the proportion of time that yearling stags spent in either “Standing” (top graph) or “Active” (lower graph) behaviour during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with NaturO™ rings (NaturO), lignocaine ring blocks (Lignocaine) or no treatment (Control) or an NaturO on one antler and Lignocaine on the other antler (Both). Correlation analysis lines for each of the treatment groups are shown.

Table 3.4.3 Correlation analyses, goodness of fit (Pearson R ²) and p-value for test for significant difference from zero for the proportion of time spent in whole body state behaviours of deer without(pre) and with (post) treatment with NaturO™ rings, lignocaine ring blocks or control. or both (NaturO on one antler, Lignocaine on other antler). Where correlations were significant they are annotated below with “Sig” and where not significant with “Not sig”					
Behaviour	Overall	Control	NaturO	Lignocaine	Both
Standing Quiet					
Pearson R ²	0.0887	0.25	0.11	0.0018	0.17
p-value	0.0034	0.0329	0.0768	0.4921	0.0873
	Sig	Sig	Not sig	Not sig	Not sig
Walking					
Spearman R	0.4541	0.5934	0.4806	0.3242	0.5562
p-value	<0.0001	0.0094	0.0072	0.0862	0.0165
	Sig	Sig	Sig	Not Sig	Sig
Pacing					
Pearson R ²	0.2272	0.0035	0.0070	0.0015	0.0108
p-value	0.0455	0.7556	0.6592	0.8407	0.3544
		Not sig	Not sig	Not sig	Not sig
Walking Backwards					
Spearman r	0.4092	0.3878	0.6441	0.5294	-0.1898
p-value	<0.0001	0.1118	0.0001	0.0031	0.4506
	Sig	Not Sig	Sig	Sig	Not sig
Circling (3)					
Spearman R	0.1379	insufficient data			
p-value	0.1712				
Lying down (4)					
Pearson R ²	insufficient data				
p-value					
Active (Walking, Pacing, Walking backwards, circling)					
Pearson R ²	0.0830	0.2418	0.0195	0.0213	0.2399
p-value	0.0036	0.0277	0.4619	0.4412	0.0284
	Sig	Sig	Not sig	Not sig	Sig
Notes					
1. Date not evenly distributed					
2. Data not evenly distributed					
3. 16 animals circled “pre”, 5 circled “post”, 2 circled on both occasions					
4. 8 animals lay down “pre” and 4 lay down “post”, 4 lay down on both occasions					

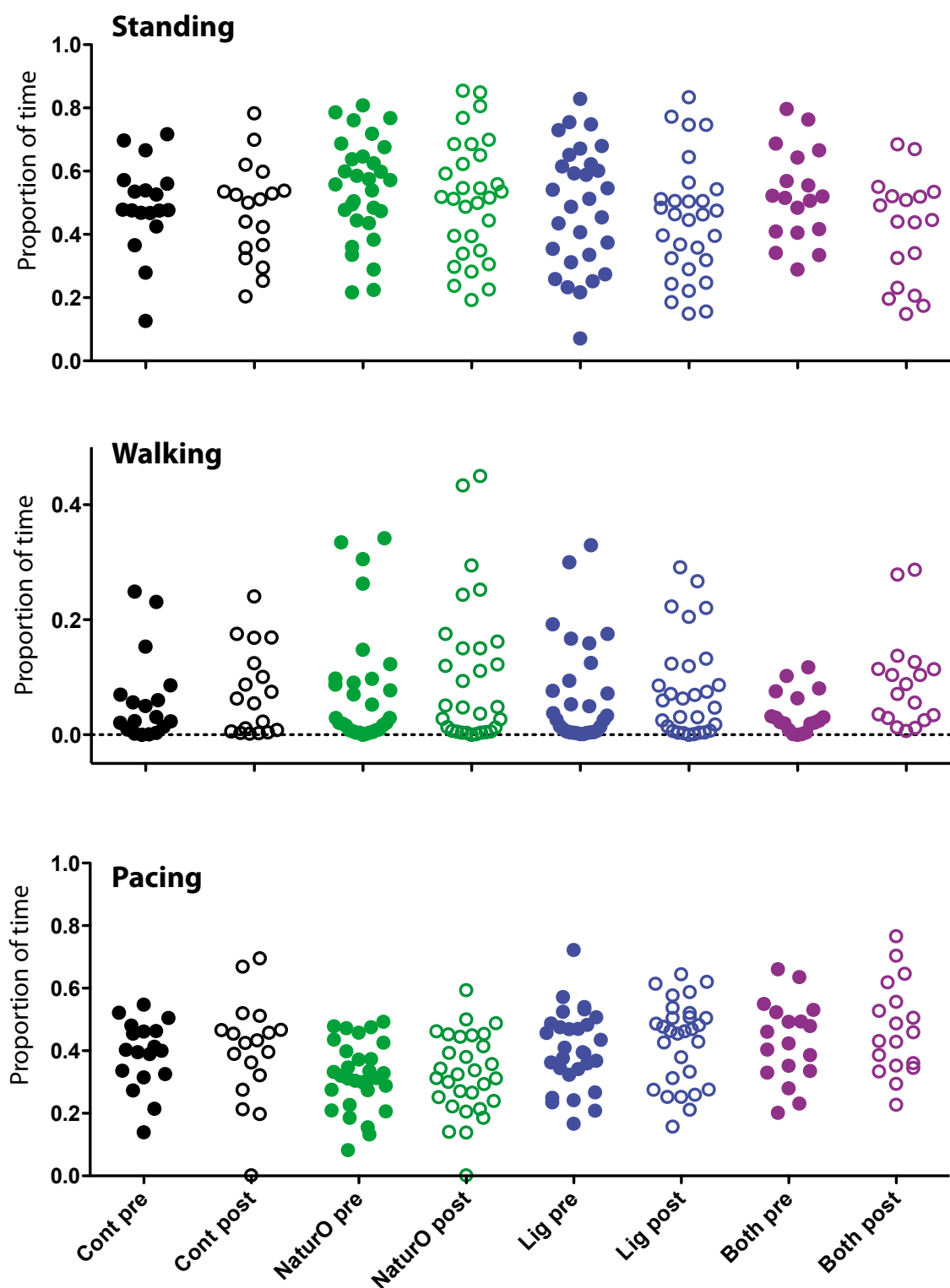


Figure 3.4.9 Individual value data plots of the proportion of time that one-year-old male deer spent in different whole body state behaviours: “Standing”, “Walking” and “Pacing” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.

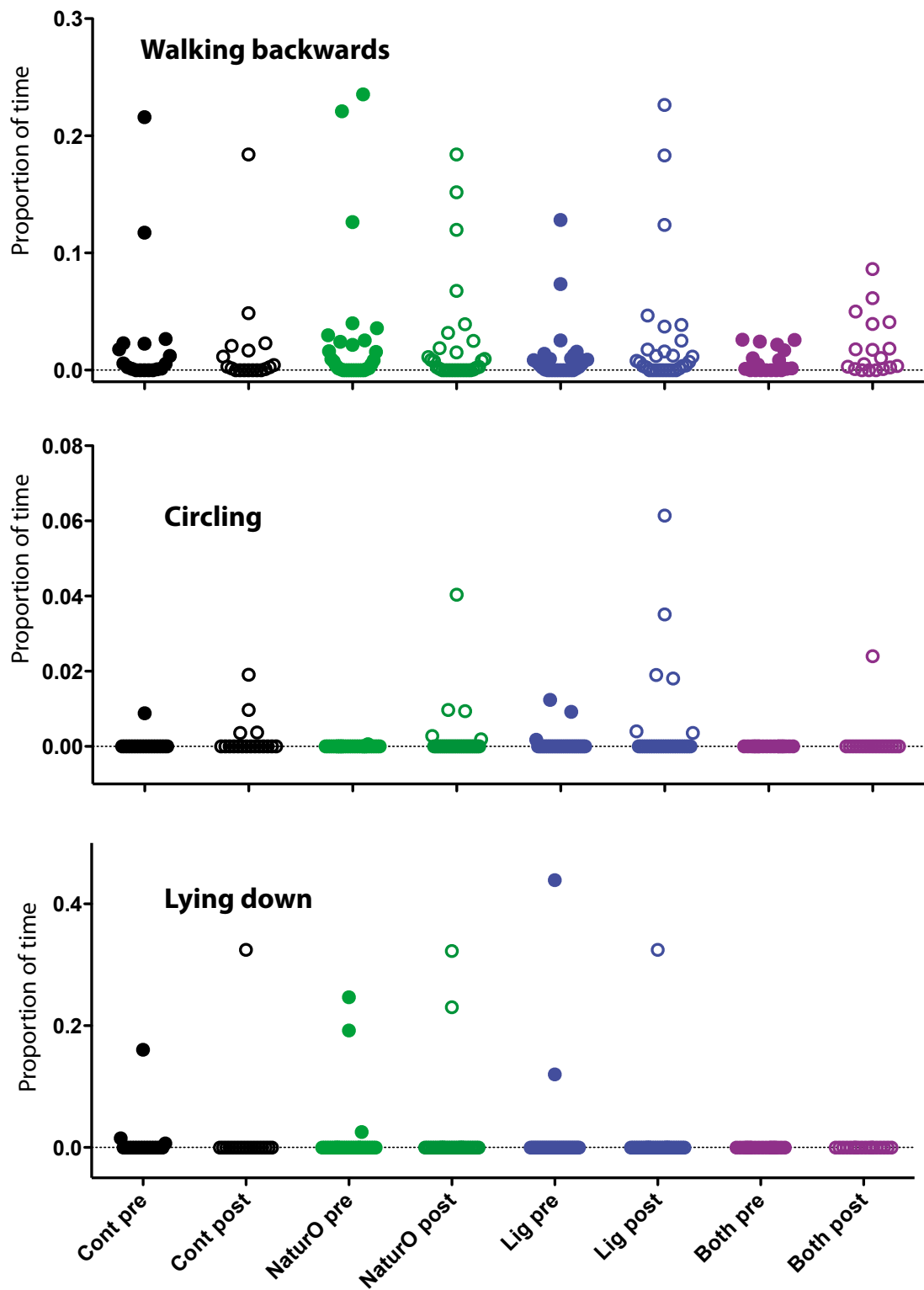


Figure 3.4.10 Individual value data plots of the proportion of time that one-year-old male deer spent in different whole body state behaviours: “Walking backwards”, “Circling” and “Lying down” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.

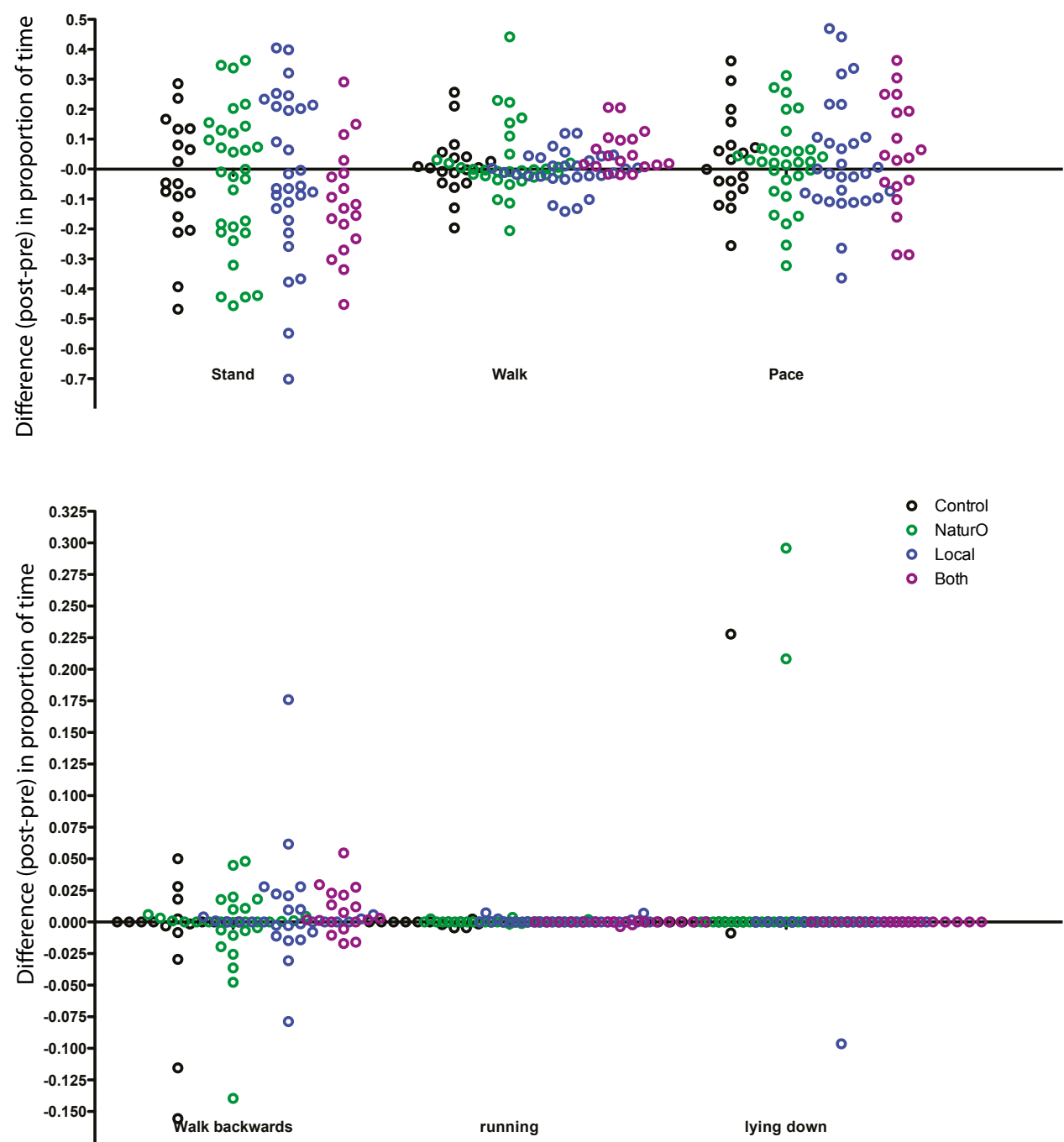


Figure 3.4.11 Individual data plot of the difference (“post” – “pre”) in the proportion of time that one-year-old male deer spent in different whole body state behaviours during one hour of video recording after treatment with either NaturO™ rings, lignocaine ring blocks or no treatment around the antler pedicle compared to no treatment.

Table 3.4.4 Number of animals that showed a 50% “increase” or “decrease” in the proportion of time spent in each whole body state behaviour when post treatment recordings were compared to pre treatment recordings. % change indicates the overall proportion of deer in the group that showed positive changes.

Behaviour	Control	NaturO	Lignocaine	Both
Standing				
No change	14	25	23	14
Increase	2	1	3	0
Decrease	2	4	3	4
% change	+0%	-10%	+0%	-22%
Walking				
No change	6	13	8	4
Increase	7	10	10	13
Decrease	5	7	11	1
% change	+11%	+10%	-3%	+67%
Walking backwards				
No change	10	14	11	5
Increase	4	9	10	9
Decrease	4	7	8	4
Overall % change	0%	+7%	+7%	+28%
Pacing				
No change	14	25	23	14
Increase	3	2	4	2
Decrease	1	3	2	2
Overall % change	+11%	-3%	+7%	+0%
Circling				
No change	15	27	23	17
Increase	2	3	4	1
Decrease	1	0	2	0
Overall % change	+6%	+10%	+7%	+6%
Lying down				
No change	16	28	28	18
Increase	1	2	0	0
Decrease	1	0	1	0
Overall % change	0%	+7%	-3%	0%

Whole body state behaviours - Time series

Figure 3.4.12 shows the mean proportion of time spent in each body position state for each 10-minute time interval during one hour of recording. Data are averages over all deer and “pre” and “post” treatment recordings are shown separately. Significant differences between “pre” and “post” are indicated.

Over time animals tended to spend less time “Standing Quiet” and more time “Walking” or “Pacing”, especially after 30 minutes. The difference over time was small. Differences between “pre” and “post” treatment recordings were significant for “Standing” during 20-30 mins ($p=0.045$), for “Pacing” during 20-30 mins ($p=0.020$) and for walking during 0-10 mins ($p=0.045$) and 50-60 minutes ($p=0.017$).

Head Position State Behaviours

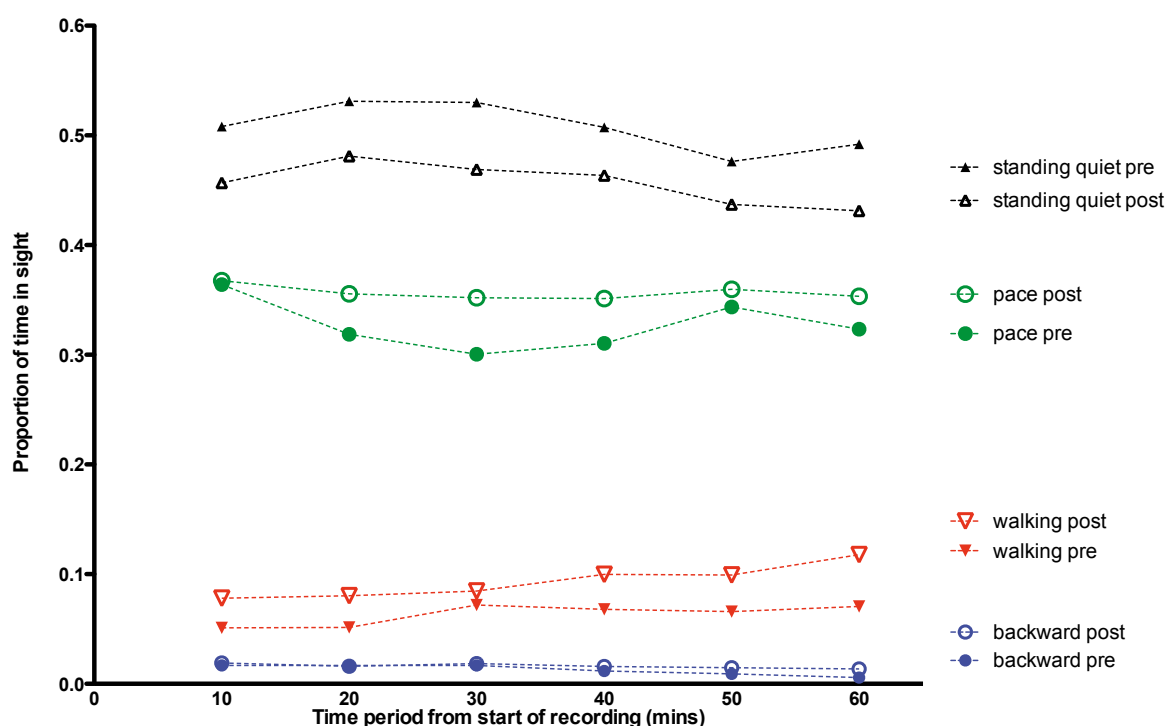


Figure 3.4.12 Time series plot of the mean proportion of time spent in four different whole body states during each 10 minute period of one hour of video observation in pens. Separate data are shown for “pre” and “post” treatment recordings. Means are for all deer across all treatment groups. Significant differences between “pre” and “post” treatment recordings are shown with an asterisk.

Head Straight

The mean proportion of time spent with the “Head straight” was 0.43 and 0.45 for “pre” and “post” treatment recordings respectively. This difference was not significant. The range was 0.17 to 0.80 and the data were approximately normally distributed. Figure 3.4.13 (top graph) shows the distribution of the proportion of time spent with “Head straight” for all animals with “pre” and “post” values shown separately.

Head Left

The mean proportion of time spent with the “Head Left” was 0.25 and 0.21 for “pre” and “post” treatment recordings respectively. This difference was not significant. The range was 0.07 to 0.44 and the data were approximately normally distributed. Figure 3.4.13 (centre graph) shows the distribution of the proportion of time spent in the “Head left” position for all animals with “pre” and “post” treatment value shown separately.

Head Right

The mean proportion of time spent with the “Head Right” was 0.21 for both “pre” and “post” treatment recordings. The range was 0.05 to 0.38 and the data was approximately normally distributed. Figure 3.4.13 (lower graph) shows the distribution of the proportion of time spent in the “Head right” position for all animals with “pre” and “post” treatment values shown separately.

Head High

The mean proportion of time spent in the “Head high” state was 0.015 and 0.012 for “pre” and “post” treatment recordings respectively. This difference was not significant. The range was 0 to 0.10 and the data was not normally distributed and was skewed to the right. Figure 3.4.14 (top graph) shows the distribution of the proportion of time spent in the “Head high” position for all animals with “pre” and “post” treatment values shown separately.

Head Low

The mean proportion of time spent in the “Head low” state was 0.086 and 0.084 for “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 0.002 to 0.40 and the data almost approximated a normal distribution. ($p > 0.1$ and $p = 0.0071$ for pre and “post” distributions respectively). Figure 3.4.14 (lower graph) shows the distribution of the proportion of time spent in the “Head low” position for all animals with “pre” and “post” treatment values shown separately.

Head position state behaviours pre vs post correlations

Figure 3.4.15 shows scatterplots of the proportion of time spent in different head position states by individual animals during the pre-treatment recording time and post-treatment recording time. Table 3.4.5 shows the goodness of fit and p-values for significant correlations for pre vs post treatment recordings of the proportion of time that each animal spent in each head state behav-

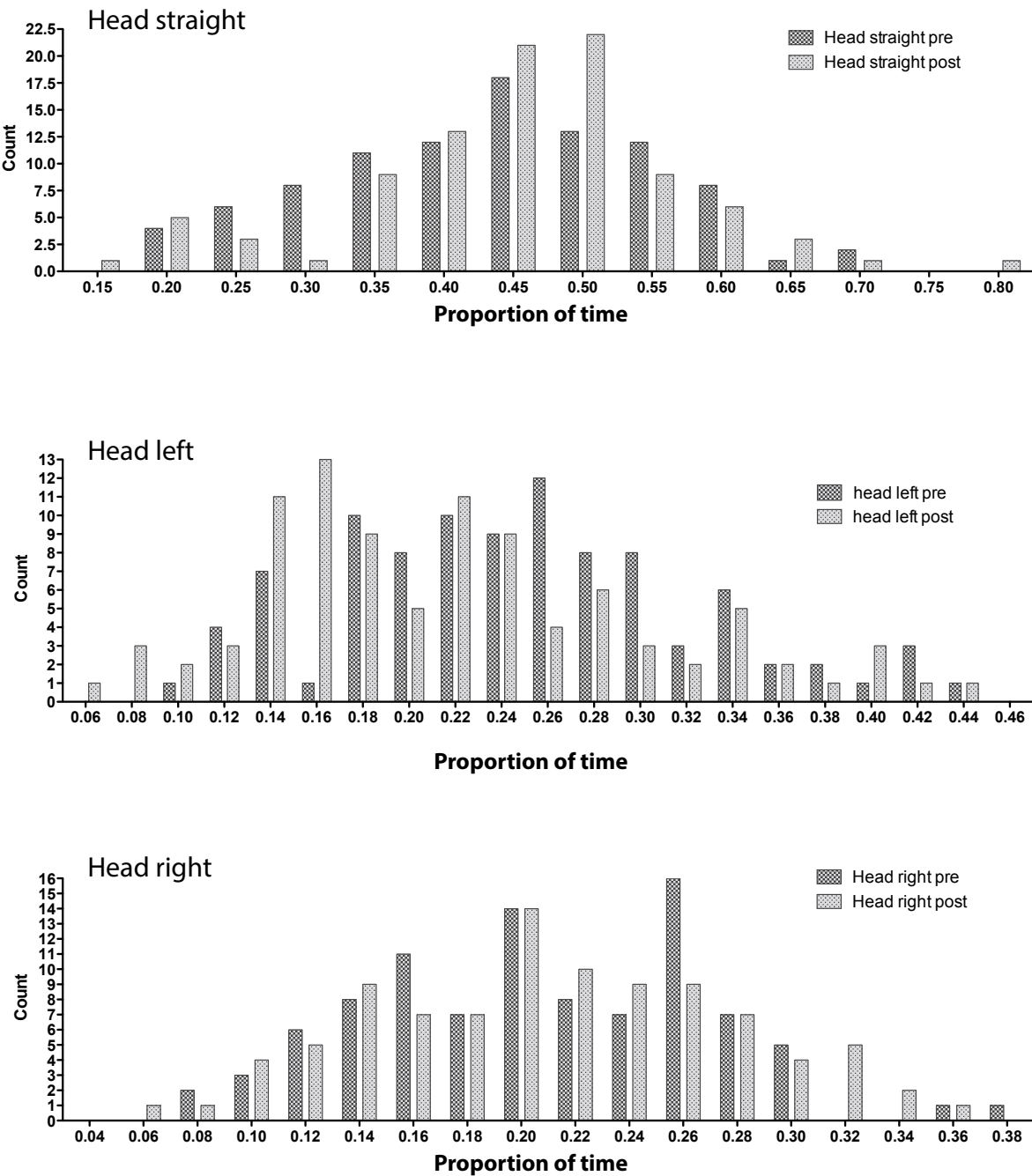


Figure 3.4.13 Histograms of the distribution of the proportion of time that one-year-old deer spent in the “Head straight” (top graph), “Head left” (centre graph) and “Head right” (lower graph) behaviour state during 60 minutes of video observation in a pen. “Pre” and “post” treatment values shown separately.

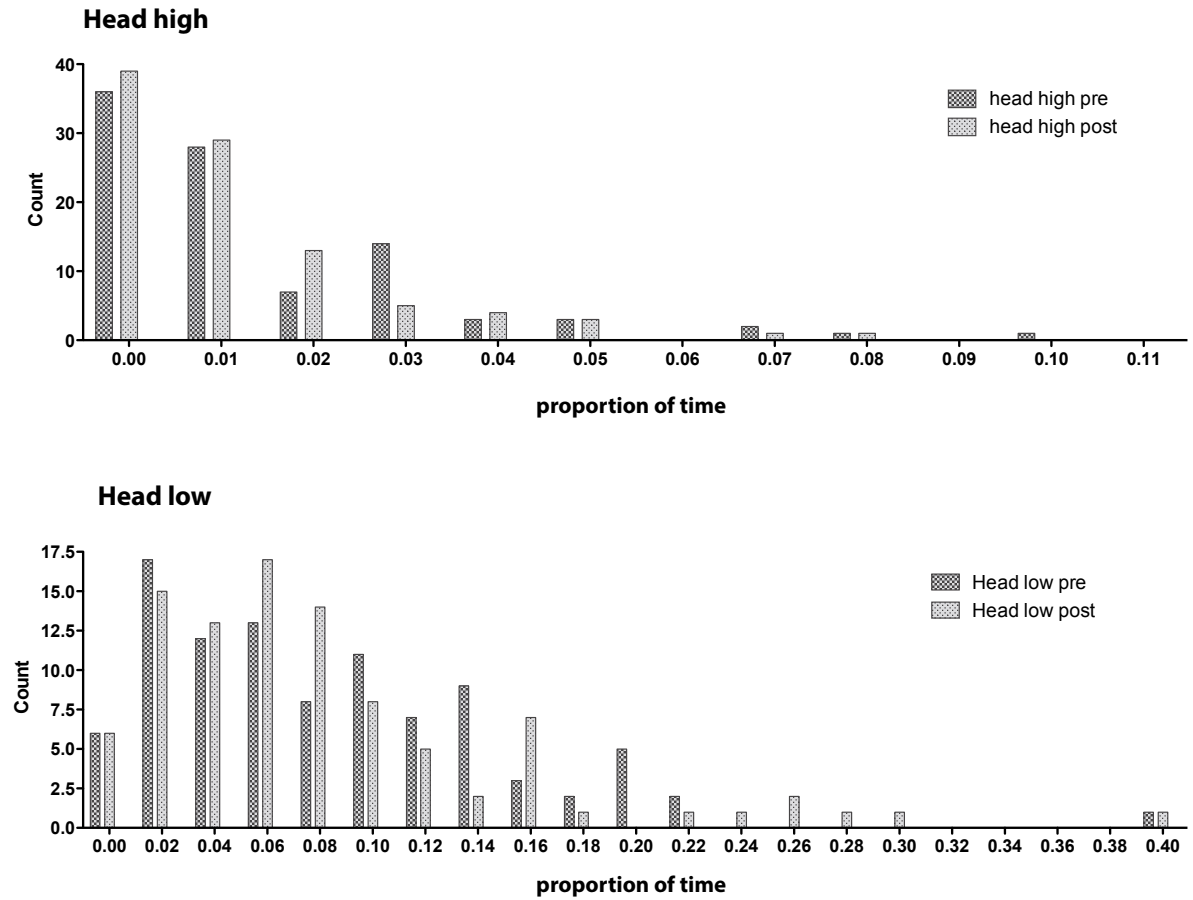


Figure 3.4.14 Histograms of the distribution of the proportion of time that one-year-old deer spent in the “Head high” (top graph), and “Head low” (lower graph) behaviour state during 60 minutes of video observation in a pen. “Pre” and “post” treatment values shown separately.

Table 3.4.5 Correlation analyses, goodness of fit (Pearson R^2) and p -value for test for significant difference from zero for the proportion of time spent in head position state behaviours of deer without(pre) and with (post) treatment with NaturO™ rings, lignocaine ring blocks or control.			
Behaviour	Pearson R^2	p -value	Comment
Head Straight	0.1004	0.0013	Only significant for control group
Head Left	0.2466	<0.0001	
Head Right	0.0109	0.3006	Not significant
Head High	0.0509	0.0240	Only significant for lignocaine group
Head Low	0.1765	<0.0001	

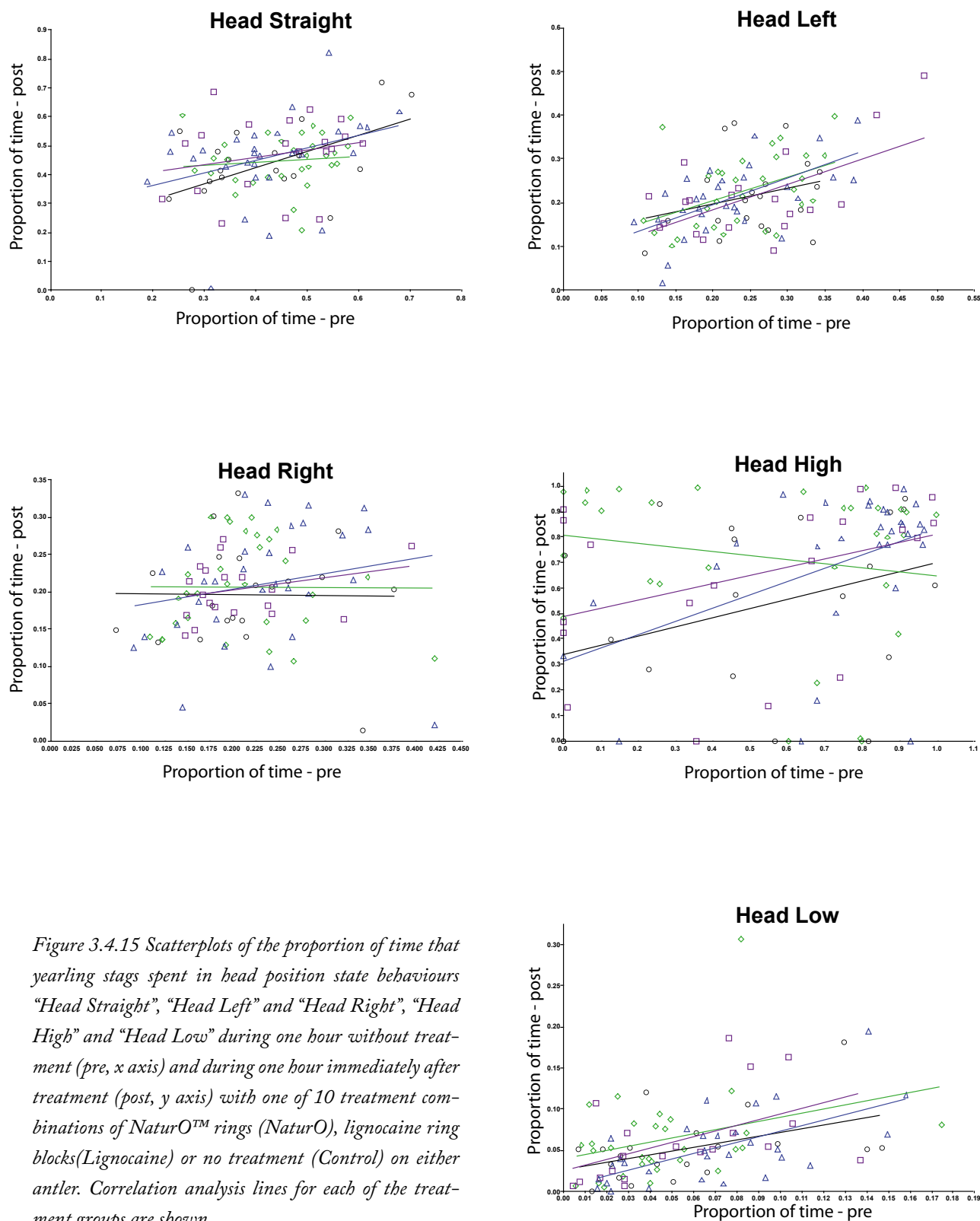


Figure 3.4.15 Scatterplots of the proportion of time that yearling stags spent in head position state behaviours “Head Straight”, “Head Left” and “Head Right”, “Head High” and “Head Low” during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with one of 10 treatment combinations of NaturO™ rings (NaturO), lignocaine ring blocks (Lignocaine) or no treatment (Control) on either antler. Correlation analysis lines for each of the treatment groups are shown.

our. There was a significant correlation in the proportion of time that individual animals spent with their “Head Left” and “Head Low” during the pre-treatment and post-treatment recordings. Pearson R values were low and the correlations were not strong.

Head position state comparison between treatment groups and comparison pre vs post.

Figures 3.4.16 and 3.4.17 show individual data plots of the proportion of time spent in each head position state during one hour of recording during the “pre” and “post” treatment periods for deer in different treatment groups. ANOVA analysis found that the means and medians did not differ significantly for any of these head state behaviours.

Within treatment group comparisons

There were no significant differences between “pre” and “post” within any of the treatments.

Between treatment group comparisons

“Pre” treatment

Control spent less time in the “Head high” position than Lignocaine

“Post” treatment

No significant differences between any groups for any of the head positions.

Difference (post-pre)

Figure 3.4.18 shows the difference between the “pre” and “post” treatment periods in the proportion of time spent in each head position state for individual deer in each treatment group. There were no significant differences between treatment groups.

Time series of head position

Figure 3.4.19 shows the mean proportion of time spent in each head position state during each ten minute period from the start of recording. Means are for all animals and pre and post treatment values are shown separately. Over time the deer tended to spend more time with their heads straight and less time in other positions. One-way ANOVA with bonferroni post test comparison showed that this difference over time was significant for “Head straight” only when comparing the 0-10 minute period with the 40 to 50 minute period and with the 50 to 60 minute period.

Proportion change for individual animals

The number of animals in each treatment group that doubled “increase”, or halved “decrease” or had “no change” in the proportion of time spent in each head position state is shown in Table 3.4.6.

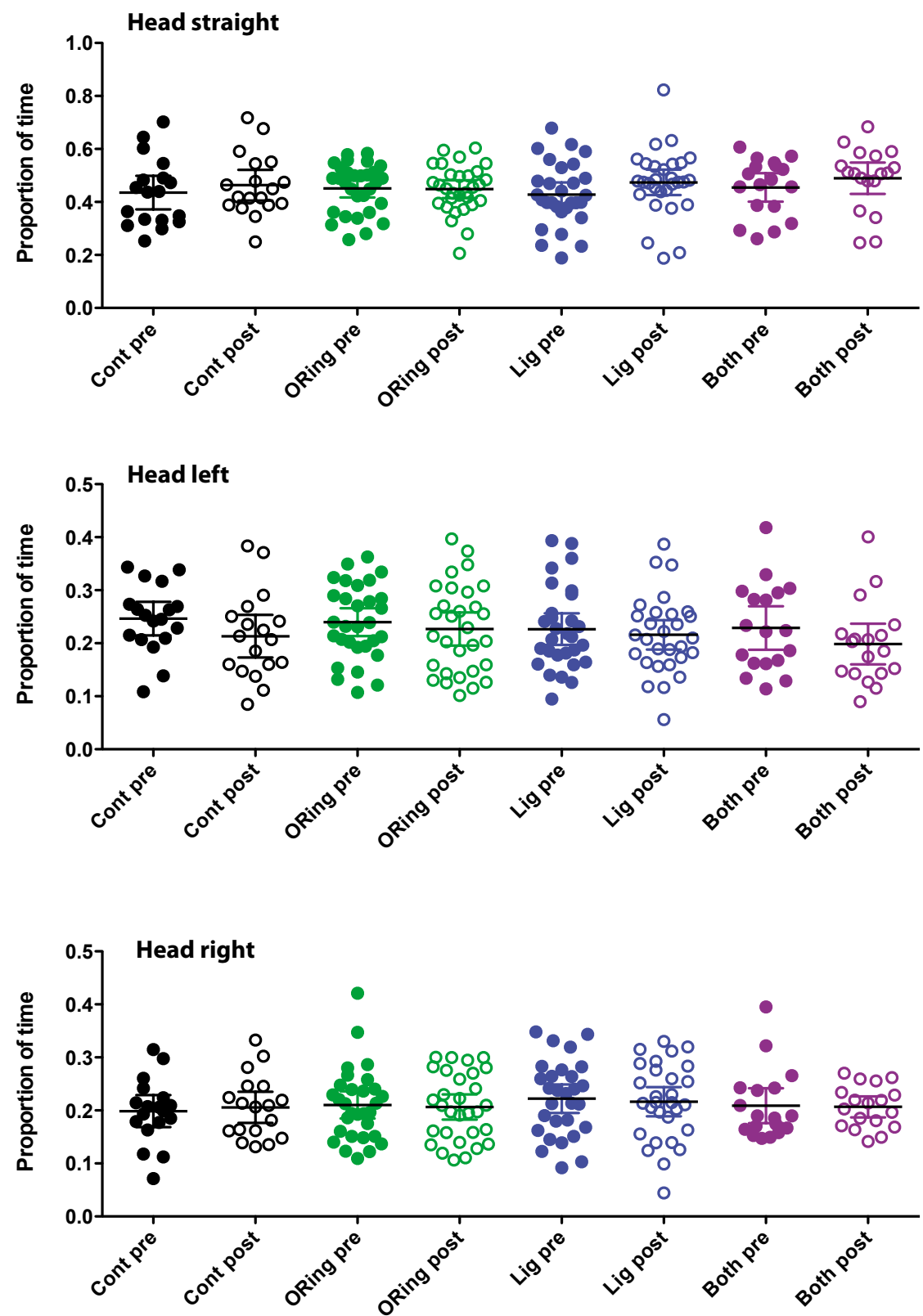


Figure 3.4.16 Individual value data plots of the proportion of time that one-year-old male deer spent in different head position state behaviours “Head straight”, “Head left” and “Head right” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.

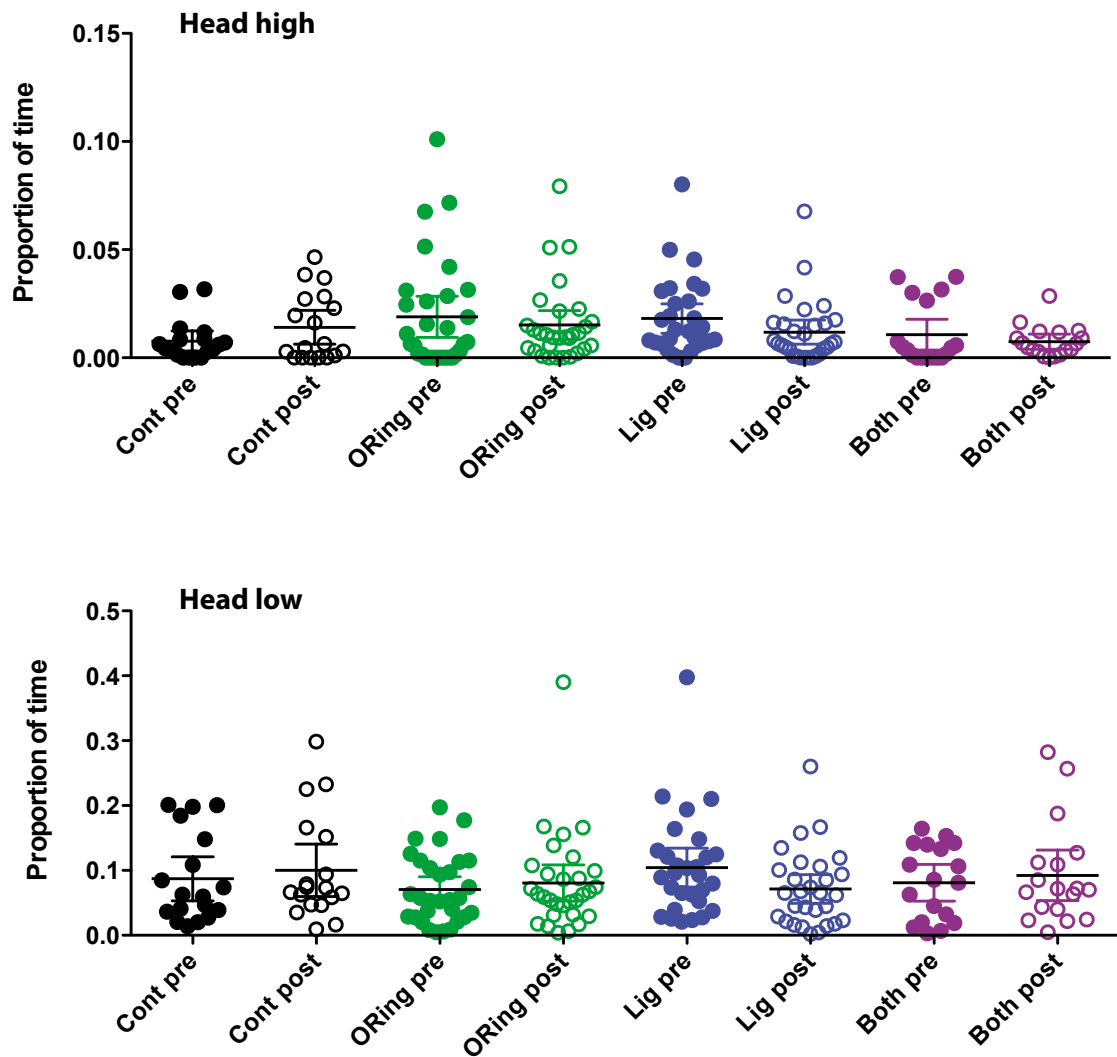


Figure 3.4.17 Individual value data plots of the proportion of time that one-year-old male deer spent in different head position state behaviours “Head high” and “Head low” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.

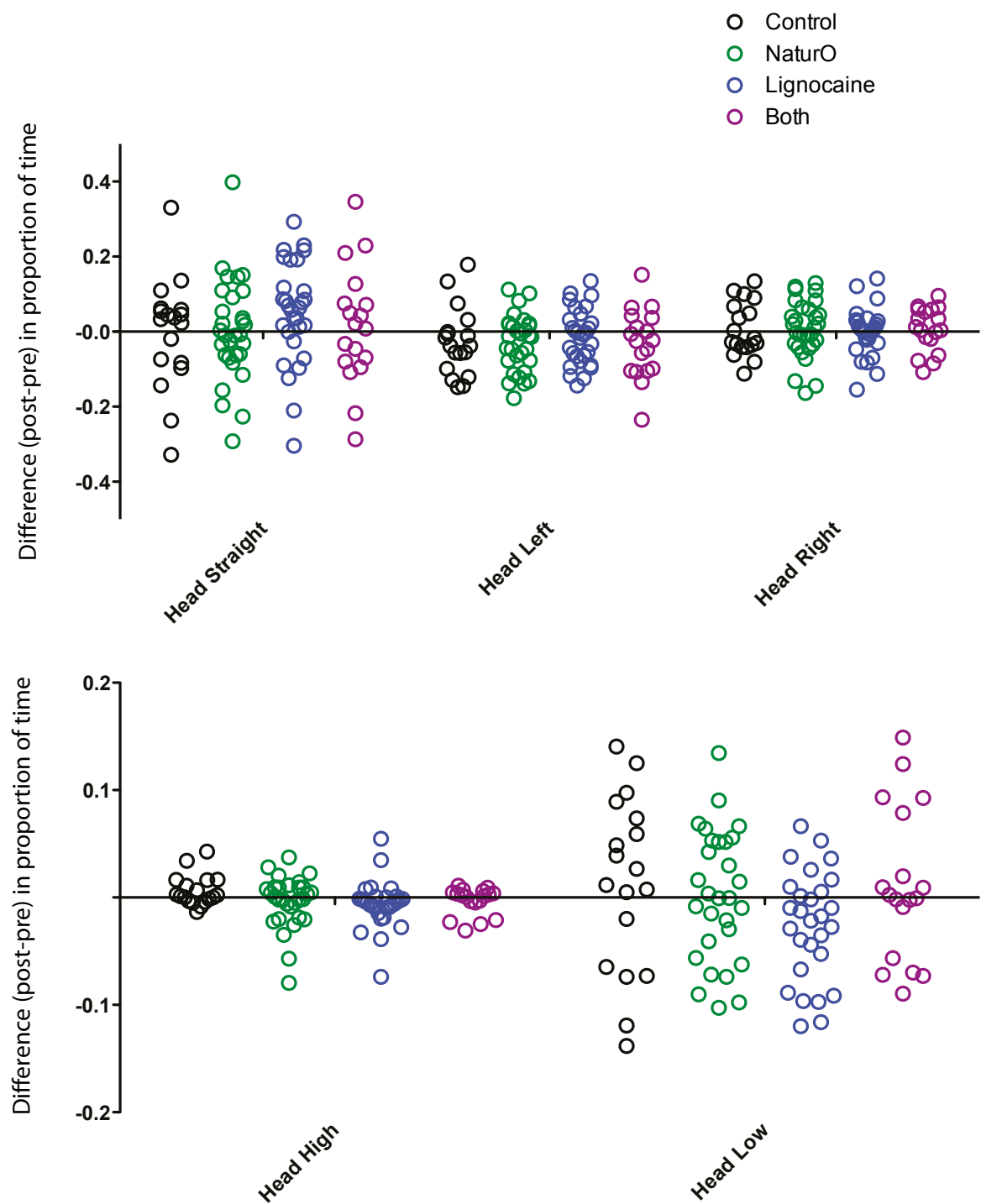


Figure 3.4.18 Individual data plot of the difference (“post” – “pre”) in the proportion of time that one-year-old male deer spent in different head position state behaviours during one hour of video recording after treatment with either NaturO™ rings, lignocaine ring blocks or no treatment around the antler pedicle compared to no treatment.

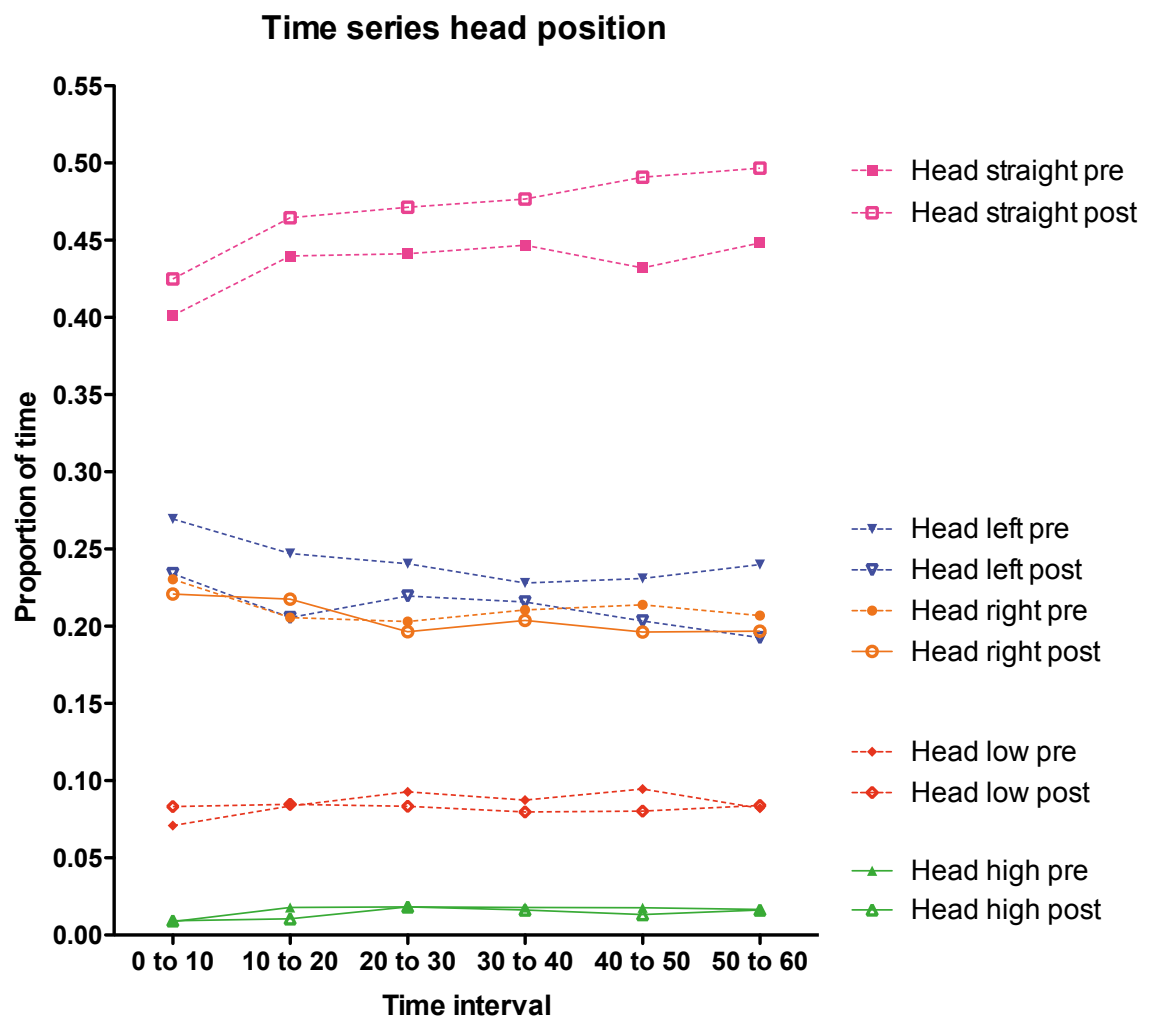


Figure 3.4.19 Time-series plot showing the proportion of time spent in each head position state for each 10 minute period of 60 minutes of video observation of deer in pens either without “pre” or following “post” treatment with NaturO™ rings, lignocaine ring blocks, no treatment or combinations of treatment.

Table 3.4.6 Number of animals that showed a 50% “increase” or “decrease” in the proportion of time spent in each head position state behaviour when post treatment recordings were compared to pre treatment recordings. % change indicates the overall proportion of deer in the group that showed positive changes.

Behaviour	Control	<i>NaturO</i>	Lignocaine	Both
Head Straight				
No change	16	28	25	14
Increase	1	1	2	2
Decrease	1	1	2	2
% change	0%	0%	0%	0%
Head Left				
No change	18	27	28	17
Increase	0	0	1	0
Decrease	0	3	0	1
% change	0%	-10%	+3%	-6%
Head Right				
No change	17	27	27	18
Increase	1	0	0	0
Decrease	0	3	2	0
% change	+6%	-10%	-7%	0%
Head High				
No change	6	7	12	2
Increase	7	13	4	10
Decrease	5	10	13	6
% change	+11%	+10%	-31%	+22%
Head Low				
No change	6	13	18	11
Increase	7	8	1	3
Decrease	5	9	10	4
% change	+11%	-3%	-31%	-6%

The “lignocaine” group had a net 31% “decrease” in “Head high” and a net 31% “decrease” in “Head low”.

There were no other significant changes.

Event Behaviours

Head Position Event

In addition to recording the proportion of time spent in each head position state, the number of times (frequency) that the animal turned its head to the “left”, “right”, “straight”, “high” or “low” was also analysed.

Data distribution

Head Straight

All deer demonstrated “Head straight” during the “pre” and “post” treatment recordings. The mean number of times that deer put their “Head straight” was 237 during the “pre” treatment recording and 250 during the “post” treatment recording. This difference was not statistically significant. The range was 69 to 268 and the data for “Head straight” were approximately normally distributed. The frequency distribution of “Head straight” is shown in Figure 3.4.20 top graph.

Head Left

All deer demonstrated “Head left” during the “pre” and “post” treatment recordings. The mean number of times that deer put their “Head left” was 136 and 133 during the “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 27 to 151 and the data were approximately normally distributed. The frequency distribution of “Head left” is shown in Figure 3.4.20 middle graph.

Head Right

All deer demonstrated “Head right” during the “pre” and “post” treatment recordings. The mean number of times that deer put their “Head right” was 132 and 140 during the “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 21 to 151 and the data were approximately normally distributed. The frequency distribution of “Head right” is shown in Figure 3.4.20 lower graph.

Head High

85/95 and 88/95 deer demonstrated “Head high” during the “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The mean number of times that deer put their “Head high” was 10.8 and 10.1 during the “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 0 to 29 and the data were not normally distributed and were skewed to the right. The frequency distribution of “Head high” is shown in Figure 3.4.21 top graph.

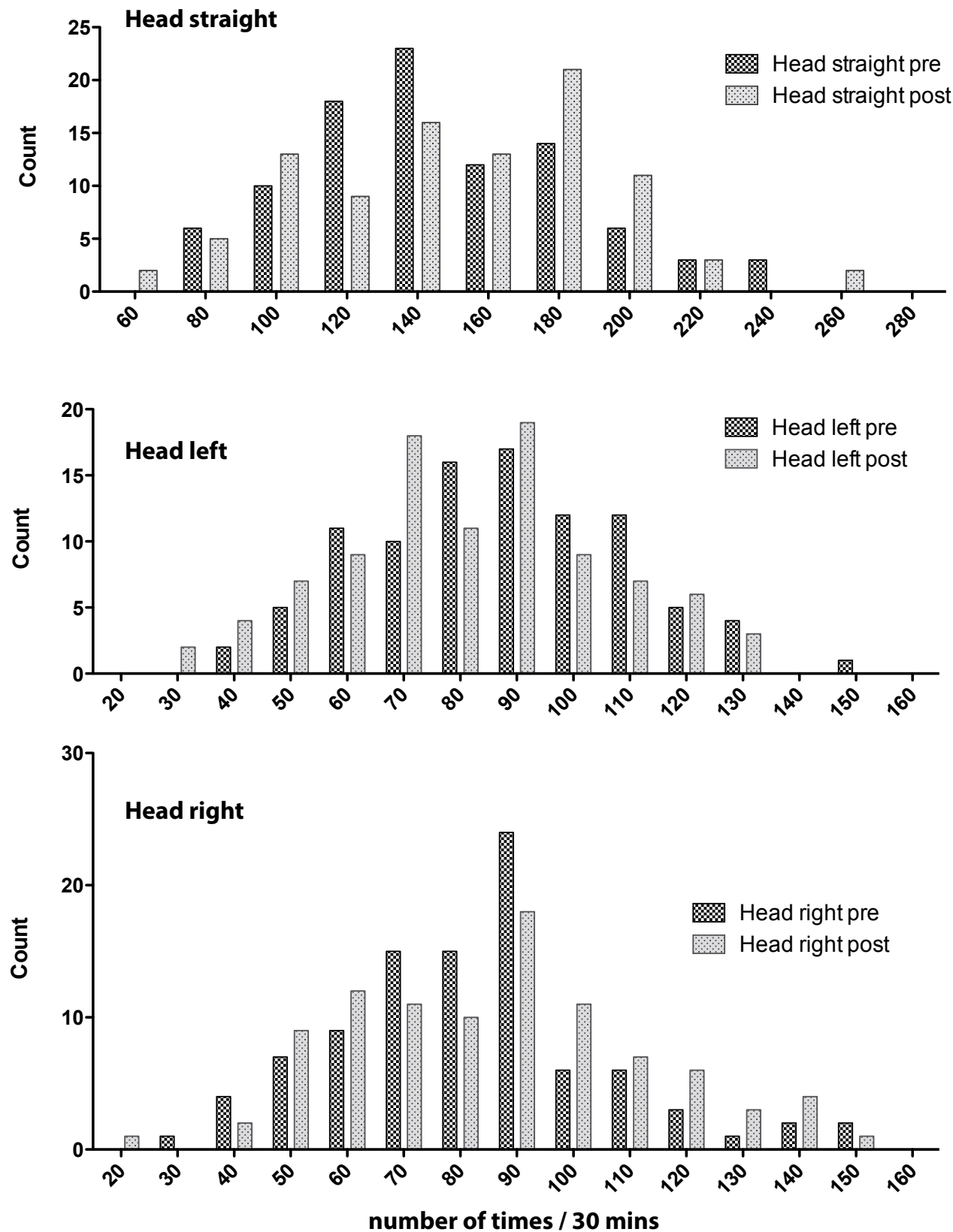


Figure 3.4.20 Histograms of the frequency distribution of the number of times one-year-old male deer put their heads in the “Head straight” (top graph), “Head left” (middle graph) and “Head right (lower graph) position during 30 minutes of video observation in a pen. “Pre” and “post” treatment values shown separately. “Count” = number of deer.

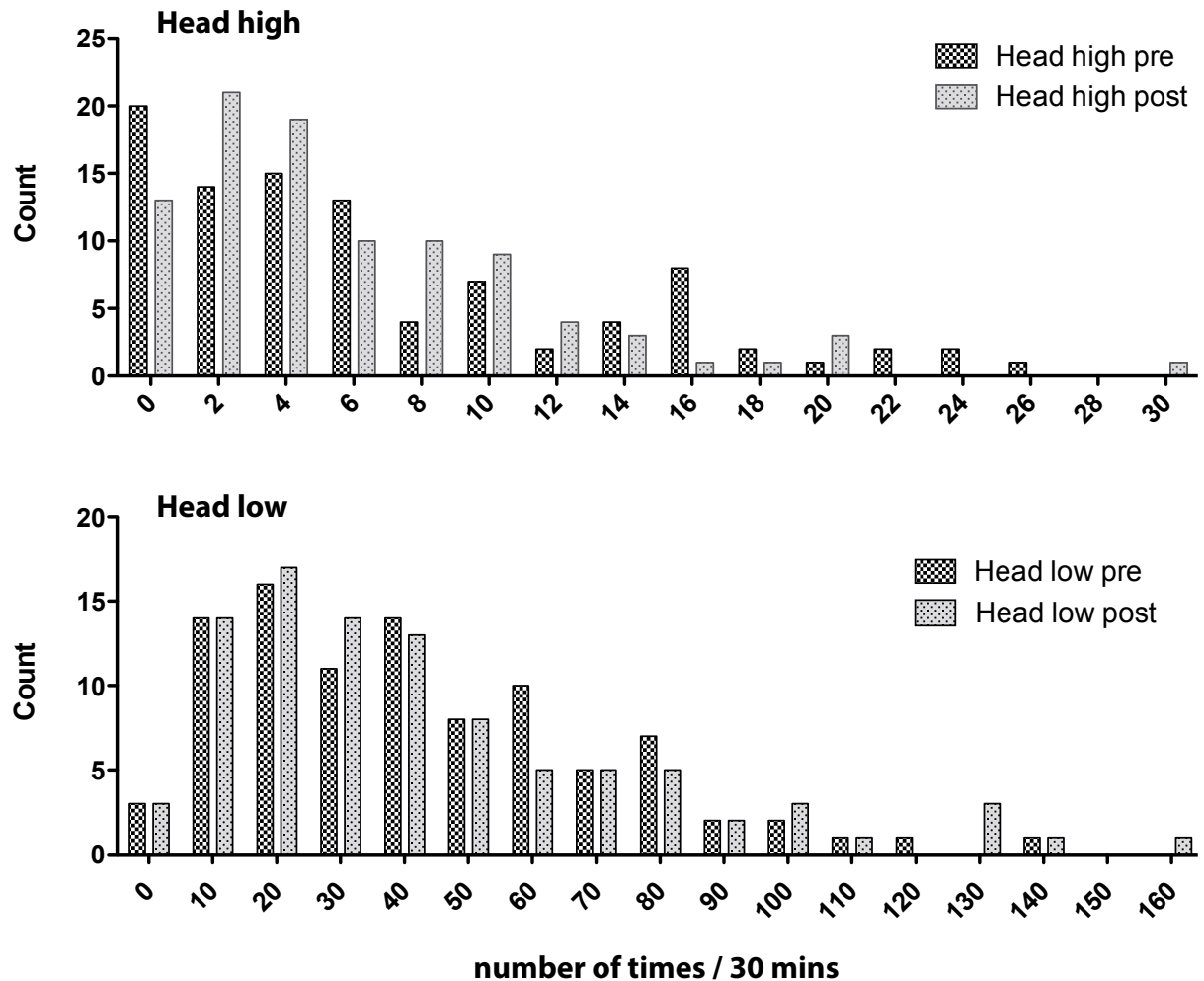


Figure 3.4.21 Histograms of the frequency distribution of the number of times one-year-old male deer put their heads in the “Head straight” (top graph), “Head left” (middle graph) and “Head right” (lower graph) position during 30 minutes of video observation in a pen. “Pre” and “post” treatment values shown separately. “Count” = number of deer.

Head Low

All deer demonstrated “Head low” during the “pre” and “post” treatment recordings. The mean number of times that deer put their “Head low” was 71 and 72 during the “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 2 to 160 and the data did not pass D’Agostino and Pearson’s omnibus normality test although the distribution was close enough to normal that comparison of the means is likely to be valid. The frequency distribution of “Head low” is shown in Figure 3.4.21 lower graph.

Pre vs Post correlation analysis

Figure 3.4.22 shows scatterplots of the number of times deer put their heads in different head positions by individual animals during the pre-treatment recording time (x-axis) and post-treatment recording time (y-axis). Table 3.4.7 shows the goodness of fit and p-values for significant correlations for pre vs post treatment recordings of the number of times that each animal put its head in each position. For data that were not normally distributed Spearman R values are shown and for data that were normally distributed the Pearson R^2 is shown. Across all animals in combined treatment groups there was a significant correlation between the “pre” and “post” treatment recordings for all head position behaviours. When individual treatment groups were examined some had significant correlations for some head positions but not others. There were no clear trends between treatment groups.

Pre vs Post comparison within groups

Figures 3.4.23 and 3.4.24 show the individual values for the number of times that each deer put its head into “Head straight”, “Head left”, “Head right”, “Head high” and “Head low” positions. Each graph shows “pre” and “post” treatment values for each of the treatment groups. ANOVA analysis showed that the means varied significantly for “Head right” only. Post ANOVA t-tests within each treatment group found the following:

Head Straight: No significant changes

Head Left: No significant changes

Head Right: Both demonstrated “Head right” more often during the “pre” treatment recording than “post” treatment. $p=0.038$.

Head high: No significant differences

Head low: No significant differences

Pre, post and diff comparisons between group

Pre Treatment

Lignocaine demonstrated “Head right” more often than *NaturO*, $p=0.0066$

Control demonstrated “Head low” more often than *NaturO*, $p=0.048$

Lignocaine demonstrated “Head low” more often than *NaturO*, $p=0.001$

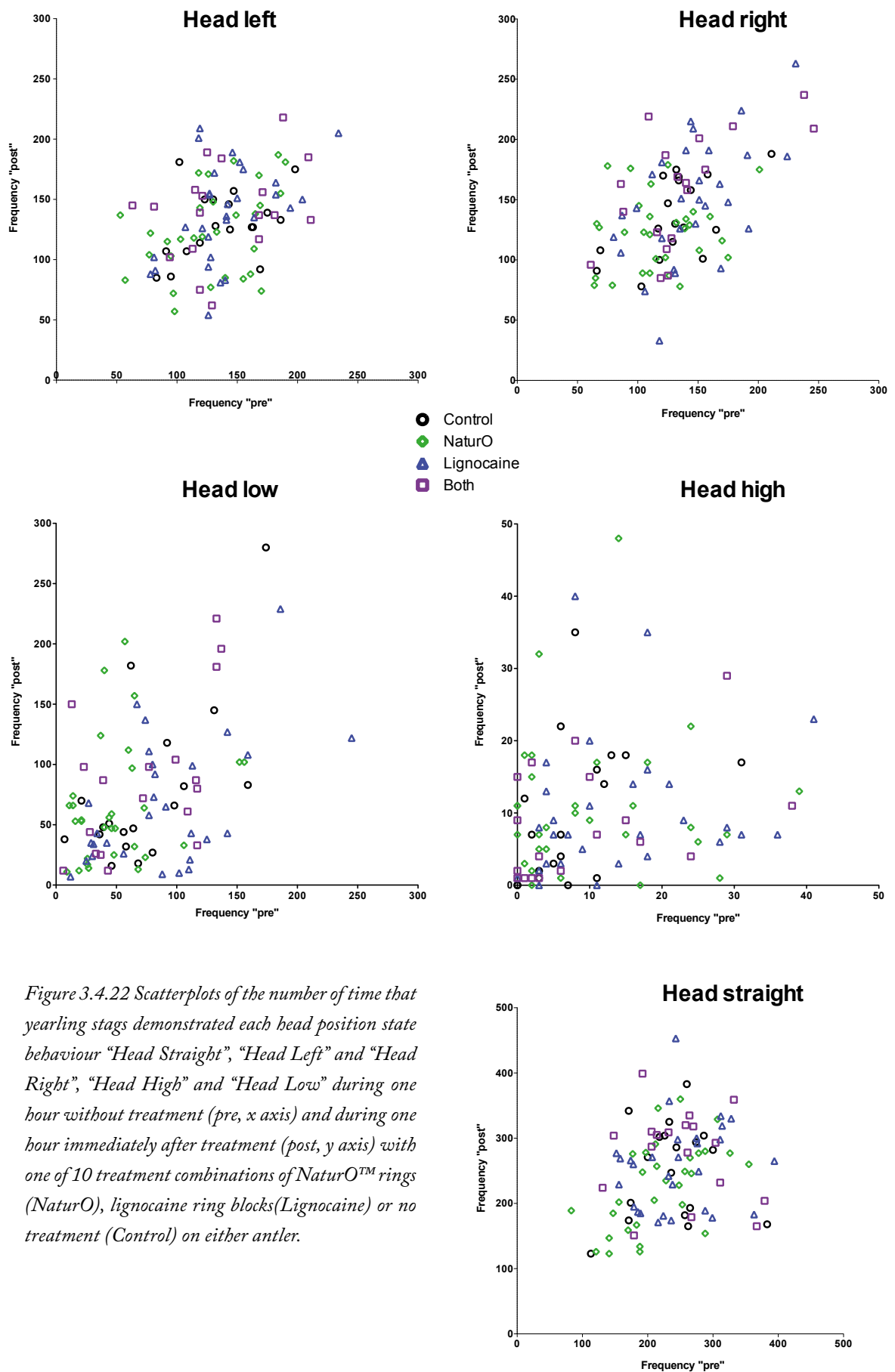


Figure 3.4.22 Scatterplots of the number of time that yearling stags demonstrated each head position state behaviour "Head Straight", "Head Left" and "Head Right", "Head High" and "Head Low" during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with one of 10 treatment combinations of NaturO™ rings (NaturO), lignocaine ring blocks (Lignocaine) or no treatment (Control) on either antler.

Table 3.4.7 Correlation analyses, goodness of fit (Pearson R^2 , Spearman R) and p -value for test for significant difference from zero for the number of times that deer demonstrated head position behaviours without (pre) versus with (post) treatment with NaturO™ rings, lignocaine ring blocks or control. or both (NaturO on one antler, Lignocaine on other antler).

Where correlations were significant they are annotated below with “Sig” and where not significant with “Not sig”

Behaviour	Overall	Control	NaturO	Lignocaine	Both
Head straight					
Pearson R^2	0.0292	0.01049	0.3070	0.02842	0.03531
p -value	0.0292	0.6860	0.0015	0.3820	0.4552
	Sig	Not Sig	Sig	Not sig	Not sig
Head left					
Pearson R^2	0.1169	0.1530	0.1435	0.2093	0.09167
p -value	0.0007	0.1085	0.0390	0.0126	0.2220
	Sig	Not Sig	Significant	Significant	Not Sig
Head right					
Pearson R^2	0.2224	0.3460	0.02661	0.2892	0.3955
p -value	< 0.0001	0.0102	0.3891	0.0026	0.0052
	Sig	Sig	Not Sig	Sig	Sig
Head high					
Spearman R	0.3607	0.5977	0.001901	0.3146	0.3771
p -value	0.0003	0.0088	0.9920	0.0965	0.1229
	Sig	Sig	Not Sig	Not Sig	Not sig
Head low					
Spearman R	0.3277	0.5129	0.1983	0.3821	0.4832
p -value	0.0012	0.0295	0.2934	0.0408	0.0422
	Sig	Sig	Not sig	Sig	Sig

Post treatment

Both demonstrated “Head straight” more often than NaturO, $p=0.019$

Lignocaine demonstrated “Head right more often than NaturO, $p=0.017$

Both demonstrated “Head right” more often than NaturO, $p=0.0028$

Difference (post-pre)

Figure 3.4.25 shows the difference between the “post” treatment frequency and the “pre” treatment frequency for each of the head position events with individual animal values shown in each treatment group.

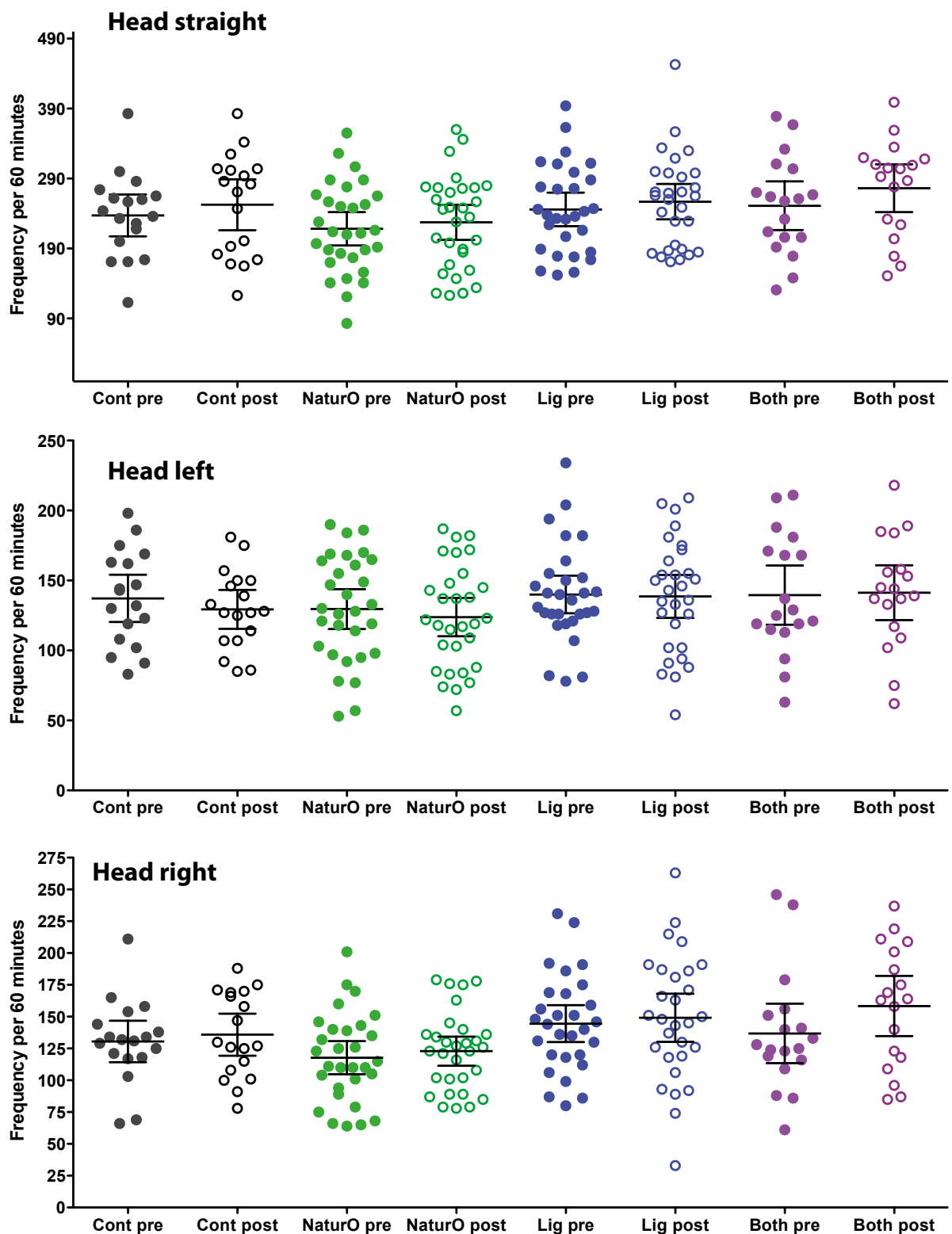


Figure 3.4.23 Individual value data plots of the number of times that one-year-old male deer demonstrated different head position behaviours “Head straight”, “Head left” and “Head right” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.

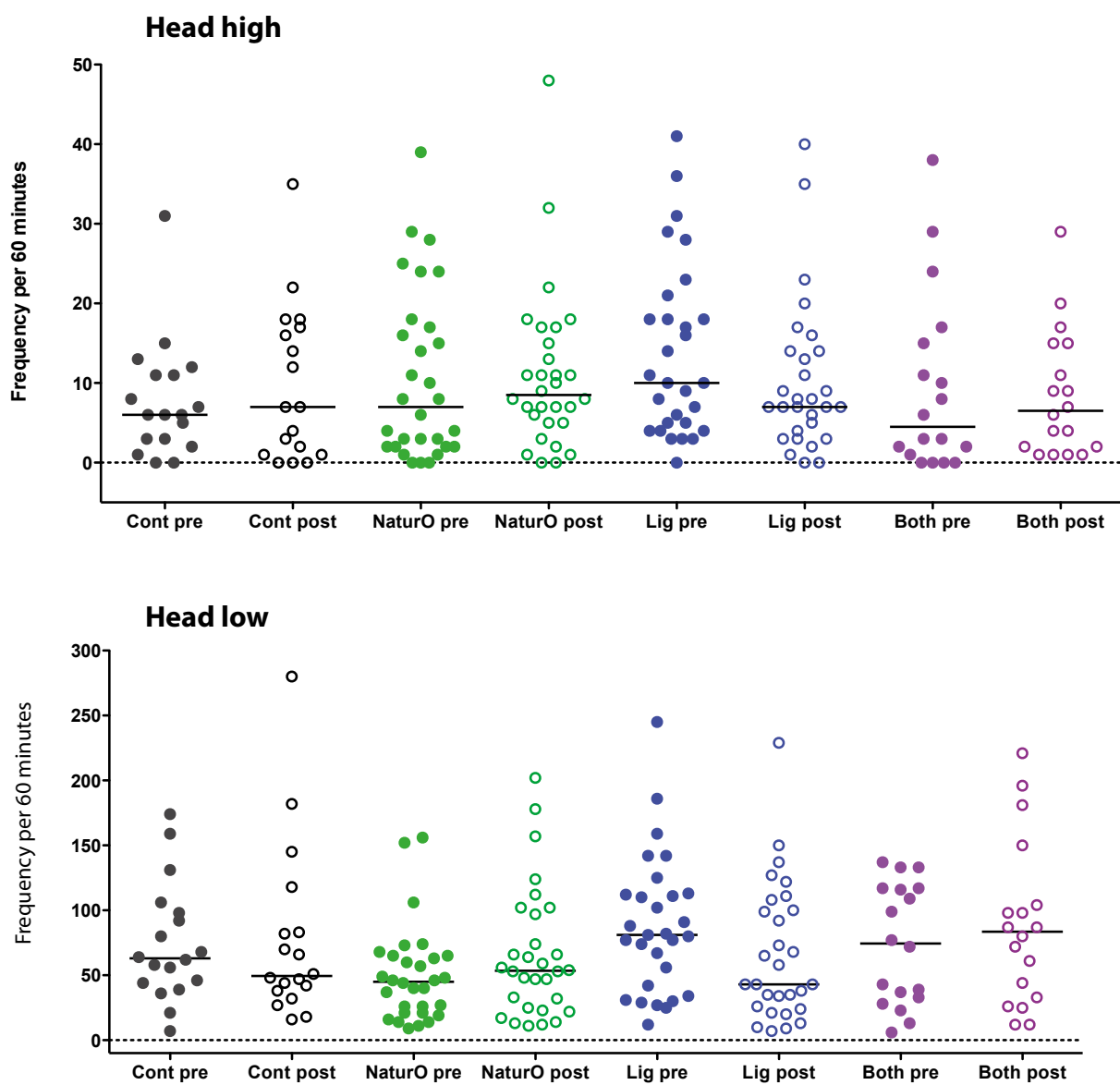


Figure 3.4.24 Individual value data plots of the number of times that one-year-old male deer demonstrated different head position behaviours “Head straight”, “Head left” and “Head right” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.

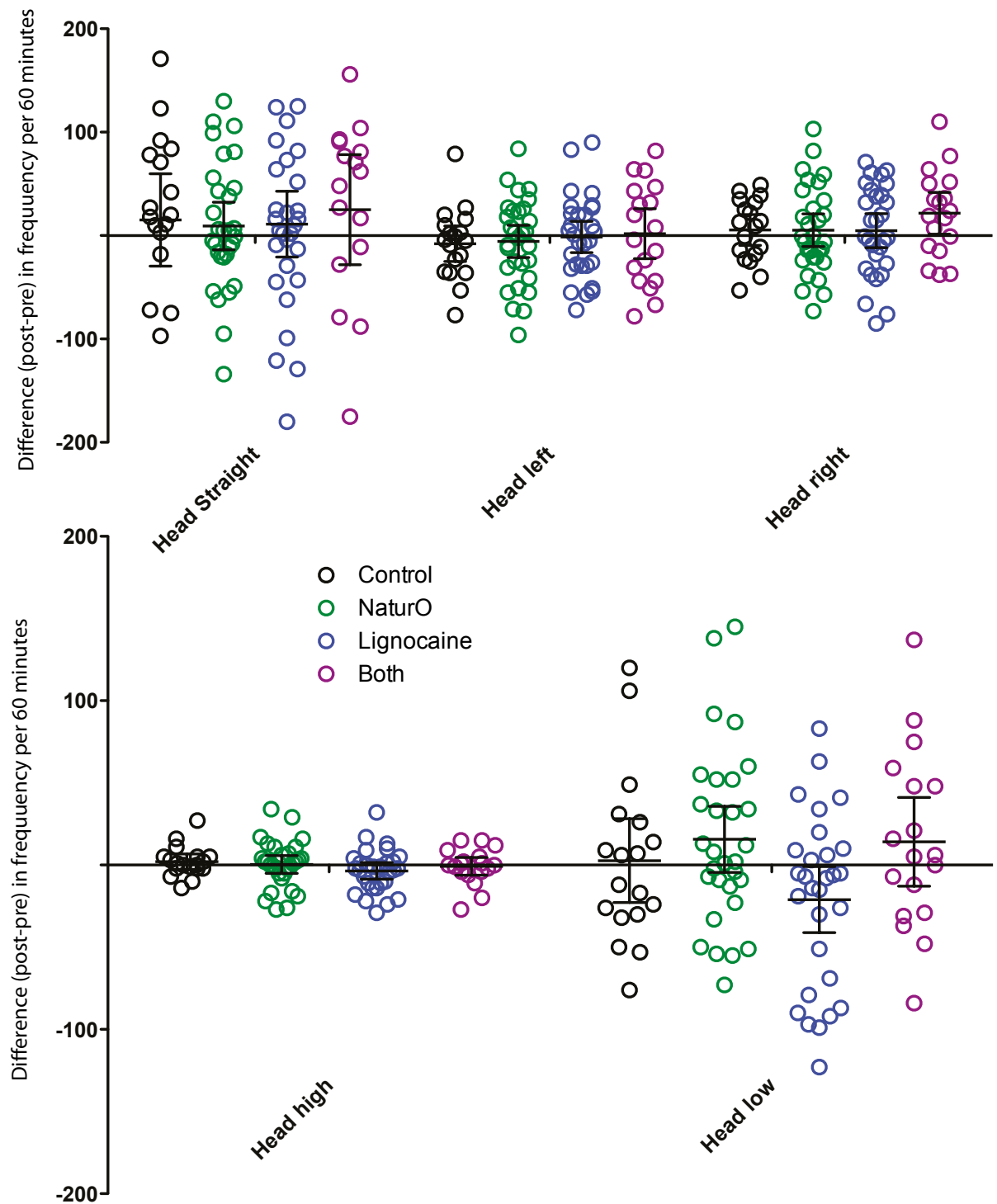


Figure 3.4.25 Individual data plot of the difference (“post” – “pre”) in the number of times that one-year-old male deer demonstrated different head position behaviours during one hour of video recording after treatment with either NaturO™ rings, lignocaine ring blocks or no treatment around the antler pedicle compared to no treatment.

Table 3.4.8 Number of animals that showed a double “increase” or halve “decrease” in the number of times they put their heads into each head position when post treatment recordings were compared to pre treatment recordings. % change indicates the overall proportion of deer in the group that showed positive changes.

Behaviour	Control	<i>NaturO</i>	Lignocaine	Both
Head Straight				
No change	17	29	28	15
Increase	0	0	0	1
Decrease	1	1	1	2
% change	-6%	-3%	-3%	-6%
Head Left				
No change	18	26	28	13
Increase	0	0	0	3
Decrease	0	4	1	2
% change	0%	-13%	-3%	+6%
Head Right				
No change	18	30	28	17
Increase	0	0	0	1
Decrease	0	0	1	0
% change	0%	0%	-3%	+6%
Head High				
No change	8	7	8	2
Increase	7	14	5	9
Decrease	4	9	16	7
% change	+11%	+17%	-38%	+11%
Head Low				
No change	9	14	19	13
Increase	4	11	1	3
Decrease	5	5	9	2
% change	-6%	+20%	-28%	+6%

One-way ANOVA and post test comparisons between treatment groups showed no significant differences between any of the treatment groups for any of the head position behaviours in the difference between the “post” treatment recordings and the “pre” treatment recordings.

Proportion of animals within groups that increased or decreased.

Table 3.4.8 shows the number of animals that had a double “increase” or halve “decrease” and the number that had “no change” in the frequency of the head position behaviours between “pre” and “post” treatment periods. The overall group change (Increase - Decrease)/Total in group is also shown. The only group that showed an overall net change of equal to or more than 30% was the “Lignocaine” group which had an overall 38% of animals net decrease in “Head high”.

Time series

Figure 3.4.26 shows the mean number of times that all deer put their heads into each of the head positions during each 10 minute interval after the start of recording. “Pre” and “Post” treatment

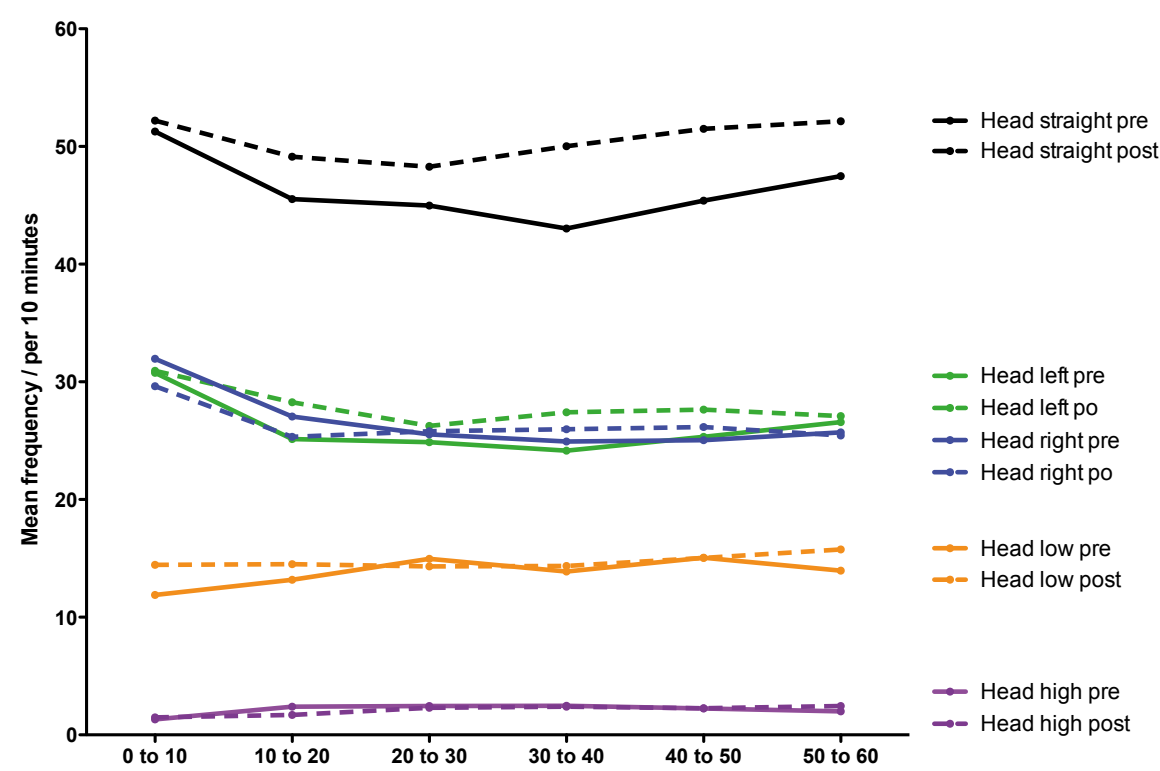


Figure 3.4.26 Time-series plot showing the mean number of time that one-year-old deer put their heads into different head positions during each 10 minute period of 60 minutes of video observation of deer in pens either without “pre” or following “post” treatment with NaturO™ rings, lignocaine ring blocks, no treatment or combinations of treatment. All treatment groups are combined.

means are shown separately. There was no clear trend with time for any of the head position behaviours although overall, deer tended to move their heads to the left, right and straight at a slightly greater frequency during the first 10 minutes.

Non-specific event behaviours

Data distribution

Look up

Thirty seven out of 95 deer demonstrated “Look up” during the “pre” treatment recordings and 31 demonstrated “Look up” during the “post” treatment recording. The mean number of times that deer “Looked up” was 2.69 and 0.78 for “pre” and “post” treatment recordings respectively. The range was 0 to 31 and the data were not normally distributed. The frequency distribution of “Look up” is shown in Figure 3.4.27 (top graph).

Nose to ground

The mean number of times that deer “Nosed the ground” was 29 and 31 for “pre” and “post” treatment periods respectively. The range was 0-125 and the data were not normally distributed and were skewed to the right. Figure 3.4.27 (middle graph) shows the frequency distribution for the number of times that deer “Nosed the ground” during the “pre” treatment and “post treatment periods.

Lick the walls

The mean number of times that deer “Licked the walls” was 13.9 and 15.3 times during the one hour of “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 0 to 75 and the data almost approximated a normal distribution. 88 and 90 out of 95 deer “Licked the walls” during the “pre” and “post” treatment recordings respectively. The frequency distribution for the number of times that individual deer “licked the walls” is shown in Figure 3.4.27 (lower graph).

Flick tongue

The mean number of times that deer “Flicked tongue” was 27 and 35 for “pre” and “post” treatment recordings respectively. This difference was significant ($p=0.0084$). The range was 1 to 171. All deer “flicked tongue” at least once. The data were almost normally distributed although it did not pass “D’Agostino & Pearson omnibus normality test”. Figure 3.4.28 (top graph) shows the frequency distribution of the number of times each deer demonstrated “Flick tongue” behaviour for the “pre” and “post” treatment periods.

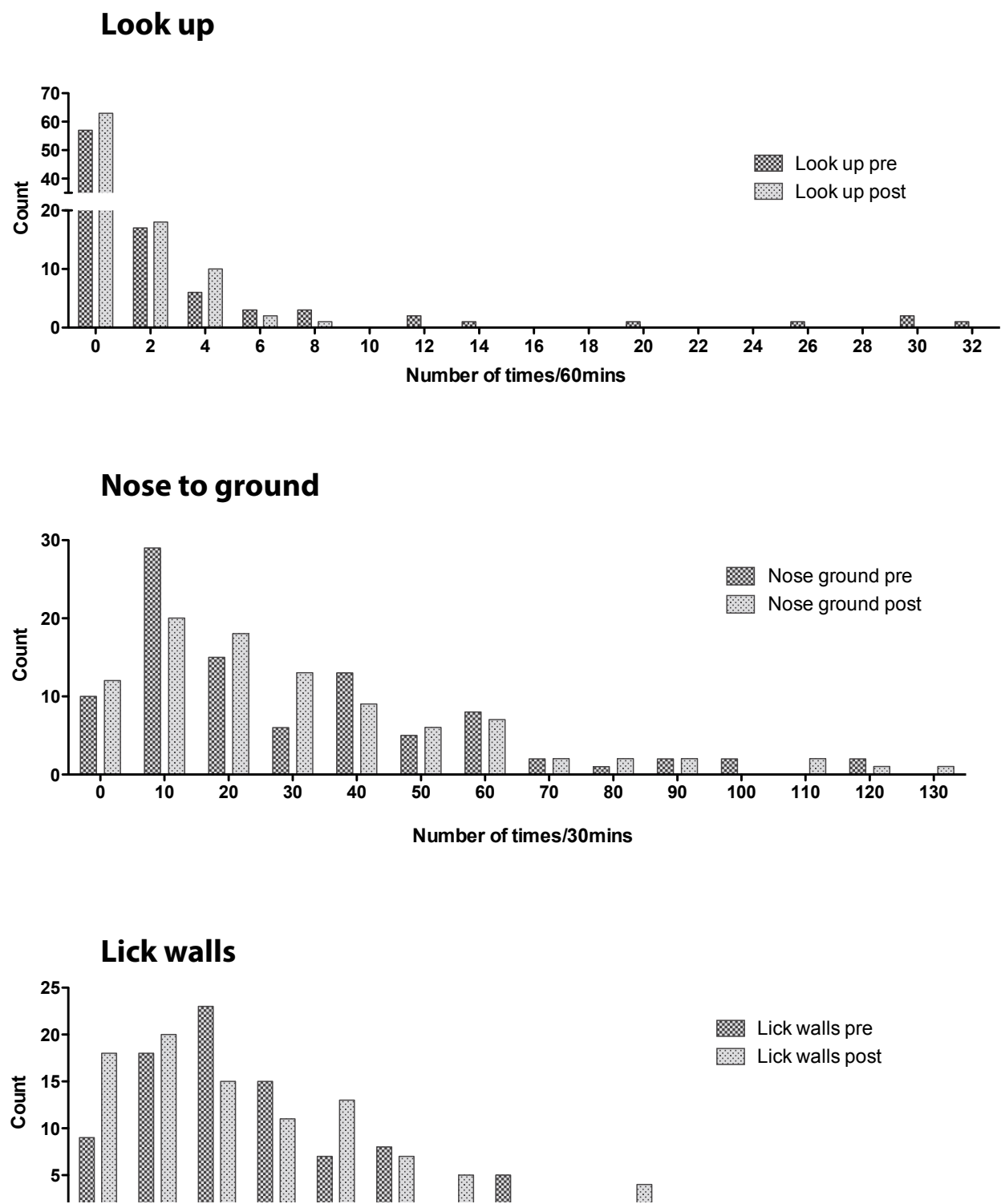


Figure 3.4.27 Histograms of the frequency distribution of the number of times one-year-old male deer demonstrated different event behaviours “Look up” (top graph), “Nose to ground” (middle graph) and “Lick walls” (lower graph) during 60 minutes of video observation in a pen. “Pre” and “post” treatment values shown separately. All treatment groups are combined. “Count” = number of deer.

Flick ears

Eighty one out of 95 and 74/95 animals demonstrated the behaviour “Flick ears” during the “pre” and “post” treatment periods respectively. This difference was not statistically significant. The mean number of times that deer demonstrated “Flick ears” was 4.4 and 4.0 for “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 0 to 31 and the data were not normally distributed. The distribution of the frequency of the behaviour “Flick ears” for each deer is shown in Figure 3.4.28 (middle graph) with “pre” and “post” shown separately.

Shake

Seventy three out of 95 and 57/95 animals demonstrated the behaviour “Shake” during the “pre” and “post” treatment periods respectively. This difference was statistically significant. The mean number of times that deer demonstrated “Shake” was 1.04 and 0.91 for “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 0 to 6 and the data were not normally distributed. The distribution of the frequency of the behaviour “Shake” for each deer is shown in Figure 3.4.28 (lower graph) with “pre” and “post” shown separately.

Groom Self

During the “pre” and “post” treatment periods 29/95 and 26/95 animals demonstrated the behaviour “Groom self” respectively. This difference was not statistically significant. The mean number of times that deer demonstrated “Groom self” was 1.0 and 0.5 for “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 0 to 31 and the data were not normally distributed and were skewed to the right. The distribution of the frequency of the behaviour “Groom self” for each deer is shown in Figure 3.4.29 (upper graph) with “pre” and “post” shown separately.

Groom other

During the “pre” and “post” treatment periods, 49/95 and 28/95 animals demonstrated the behaviour “Groom other” respectively. This difference was statistically significant ($p=0.0228$). The mean number of times that deer demonstrated “Groom other” was 2.2 and 0.53 for “pre” and “post” treatment recordings respectively. This difference was statistically significant. The range was 0 to 24 and the data were not normally distributed and were skewed to the right. The distribution of the frequency of the behaviour “Groom other” for each deer is shown in Figure 3.4.29 (middle graph) with “pre” and “post” shown separately.

Scratch

During the “pre” and “post” treatment periods 13/95 and 10/95 animals demonstrated the behav-

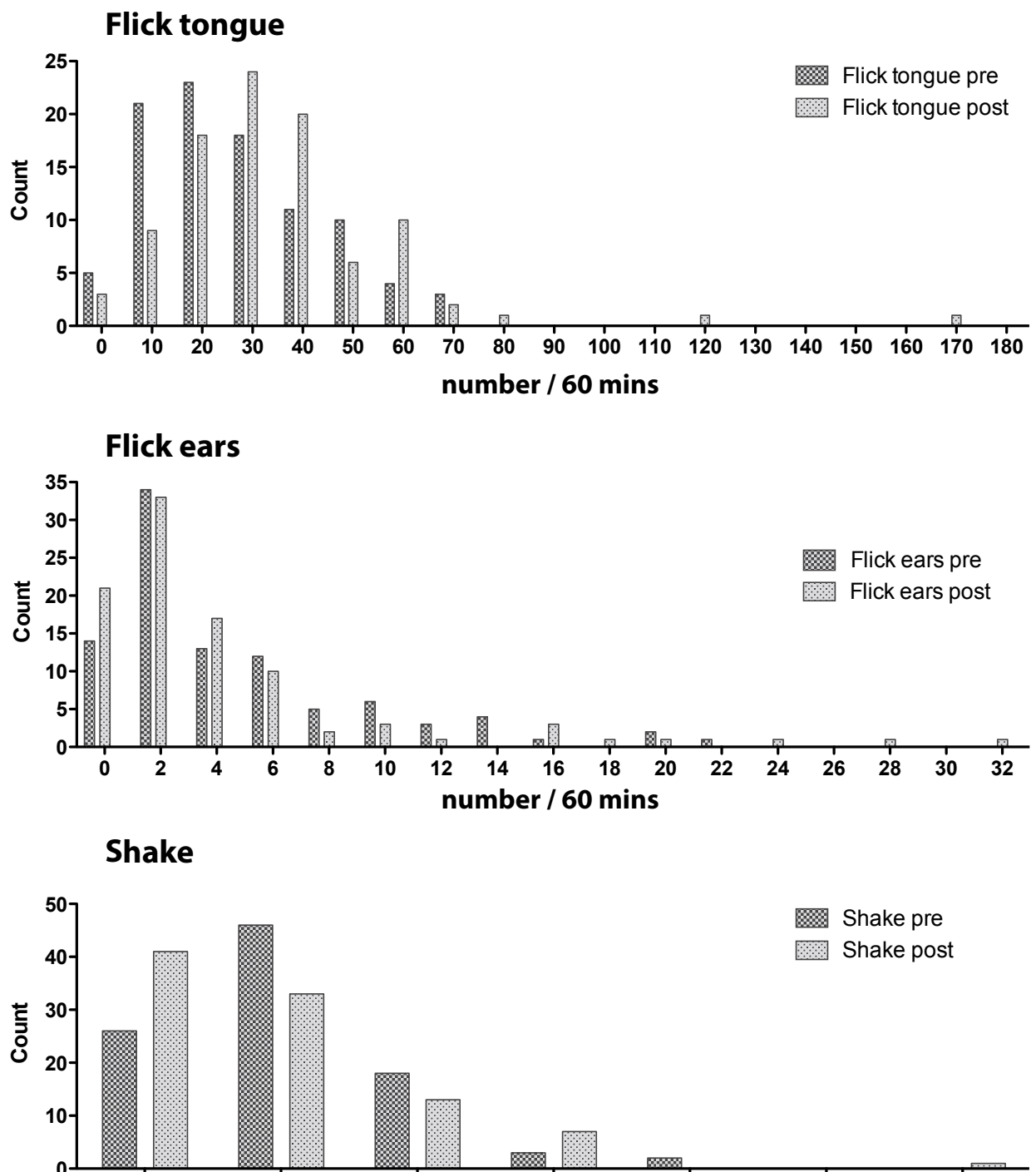


Figure 3.4.28 Histograms of the frequency distribution of the number of times one-year-old male deer demonstrated different event behaviours “Flick tongue” (top graph), “Flick ears” (middle graph) and “Shake” (lower graph) during 60 minutes of video observation in a pen. “Pre” and “post” treatment values shown separately. All treatment groups are combined. “Count” = number of deer.

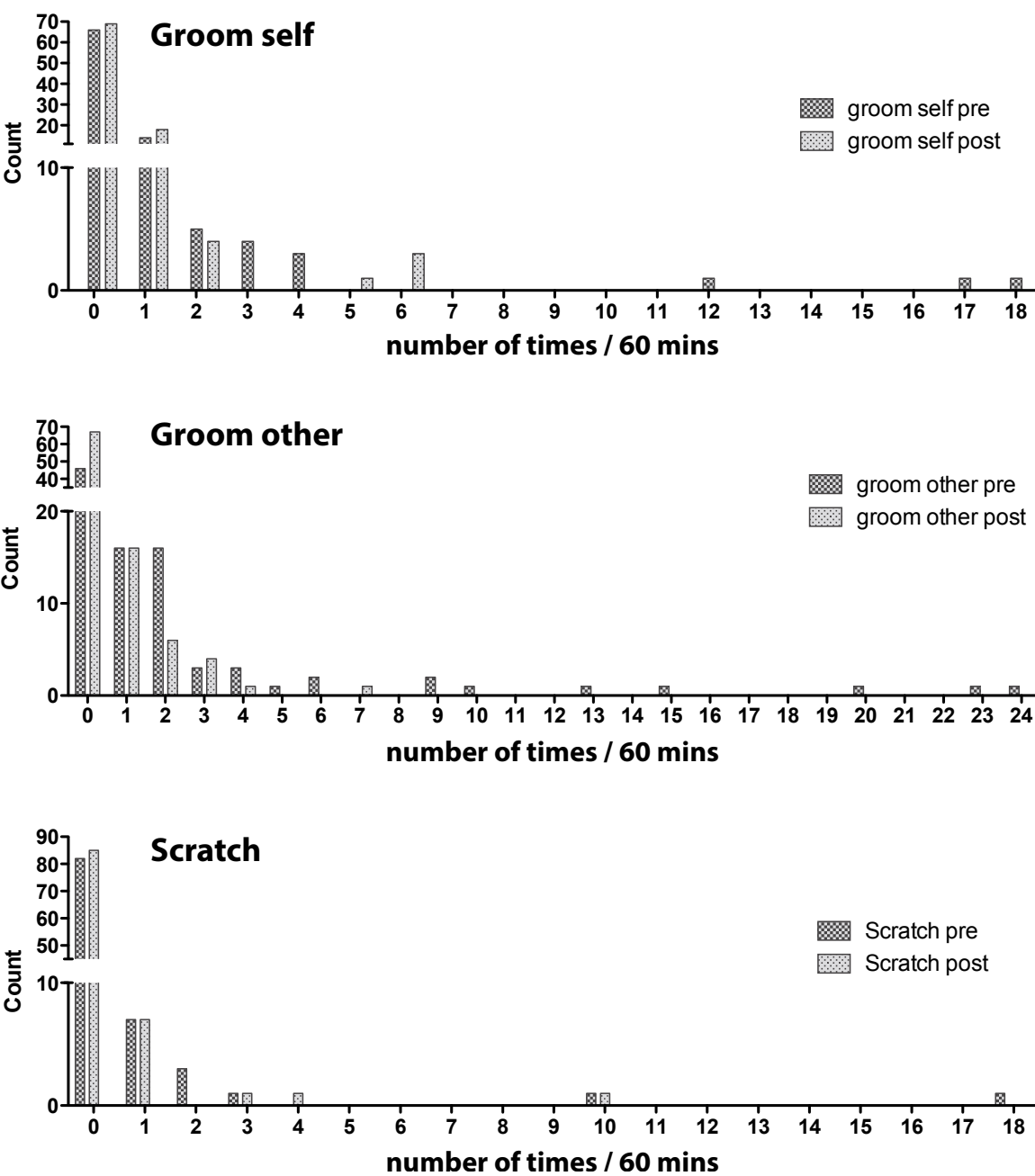


Figure 3.4.29 Histograms of the frequency distribution of the number of times one-year-old male deer demonstrated different event behaviours “Groom self” (top graph), “Groom other” (middle graph) and “Scratch” (lower graph) during 60 minutes of video observation in a pen. “Pre” and “post” treatment values shown separately. All treatment groups are combined. “Count” = number of deer.

your “Scratch” respectively. This difference was not statistically significant. The mean number of times that deer demonstrated “Scratch” was 0.46 and 0.25 for “pre” and “post” treatment recordings respectively. This difference was not statistically significant. The range was 0 to 18 and the data were not normally distributed and were skewed to the right. This behaviour was not analysed further. The distribution of the frequency of the behaviour “Scratch” for each deer is shown in Figure 3.4.29 (lower graph) with “pre” and “post” shown separately.

Fall Down

One deer fell down during observation. This behaviour was not analysed.

Bite or Bitten by Other

Two deer bit other deer or were bitten by another deer during the “pre” treatment period and three deer bit another deer or were bitten during the “post” treatment period. These were insufficient numbers to analyse.

Pre versus Post correlations

“Pre” versus “Post” scatterplots for individual animals are shown in Figures 3.4.30 (“Look up”, “Nose to ground”, “Lick the walls”), 3.4.31 (“Flick tongue”, “Flick ears”, “Shake”) and 3.4.32 (“Groom self”, “Groom other”, “Scratch”). Different treatment groups are indicated by different coloured symbols. A table of the correlation analysis is provided in Table 3.4.9.

There was a significant correlation between the “pre” and “post” treatment recordings for “Nose to ground” and “Flick tongue” for all treatment groups. There was a significant correlation for “Flick ears” in the Control and Lignocaine groups and overall.

For all other non-specific event behaviours there was one or fewer treatment groups that showed a significant correlation between the “pre” and “post” treatment recording.

Pre vs Post comparison within groups

Figures 3.4.33 to 3.4.35 show individual animal data plots of the frequency of non-specific event behaviours during the “pre” and “post” treatment periods for each treatment group. ANOVA was used to test for significant difference between the medians across all groups and individual Wilcoxin matched pairs sign ranked tests were used to compare medians within treatments.

Look up

In the Control group “Look up” was demonstrated more often during the “pre” treatment period than the “post” treatment period ($p=0.041$). There were no significant differences for other treat-

ment groups.

Nose go ground

There were no significant differences between the “pre” and “post” treatment frequencies of “Nose the ground” for any of the treatment groups

Lick the walls

There were no significant differences between the “pre” and “post” treatment means for “Lick the walls” for any of the treatment groups.

Flick tongue

Deer in the “Control” group demonstrated “Flick tongue” at a significantly higher frequency during the “post” treatment period than the “pre” treatment period ($p=0.0056$)

Deer in the *NaturO* group demonstrated “Flick tongue” at a significantly higher frequency during the “post” treatment period than during the “pre” treatment period ($p=0.035$).

Flick ears

There were no significant differences between the “pre” and “post” treatment means of “Flick ears” within any of the treatment groups. In the “Lignocaine” group the difference approached significance ($p=0.0501$) with the “post” treatment mean lower than the “pre” treatment mean.

Shake

Deer in the “*NaturO*” group demonstrated “Shake” less frequently during the “post” treatment period than the “pre” treatment period ($p=0.001$). There were no other differences within any other treatment groups

Groom self

Deer in the Lignocaine group demonstrated “Groom self” more often during the “pre” treatment period than the “post” treatment period ($p=0.0078$). There were no significant differences within the other treatment groups.

Groom other

Deer in the Lignocaine group demonstrated “Groom other” more often during the “pre” treatment

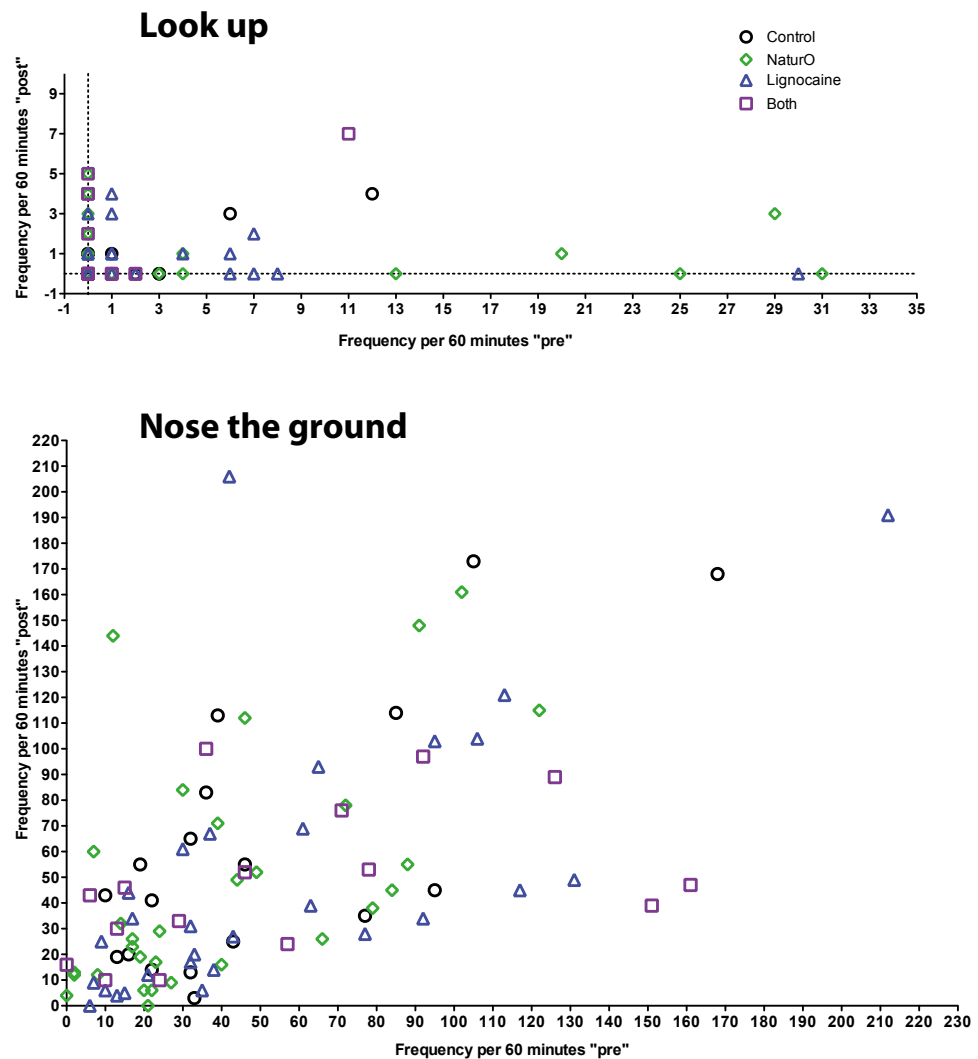
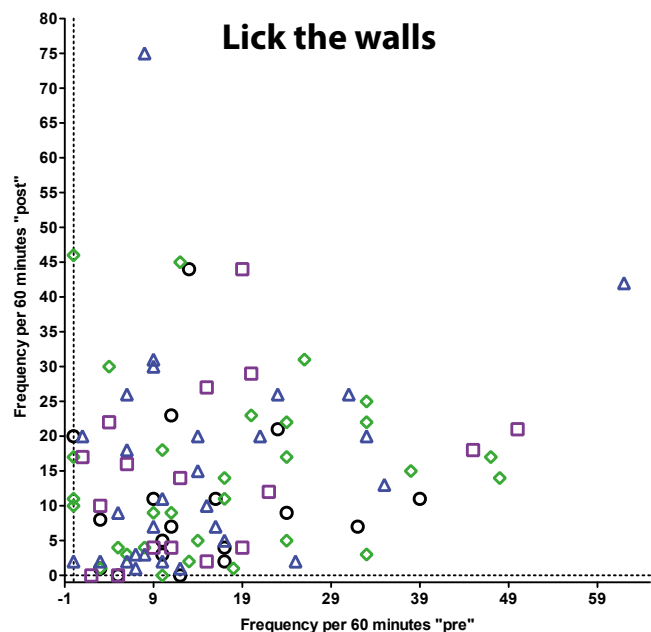


Figure 3.4.30 Scatterplots of the number of time that yearling stags demonstrated the event behaviours "Look up" (top graph), "Nose to ground" (middle graph) and "Lick the walls" (lower graph) during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO™ rings (NaturO), lignocaine ring blocks (Lignocaine) no treatment (Control) or a NaturO™ ring on one antler and lignocaine on the other antler (Both).



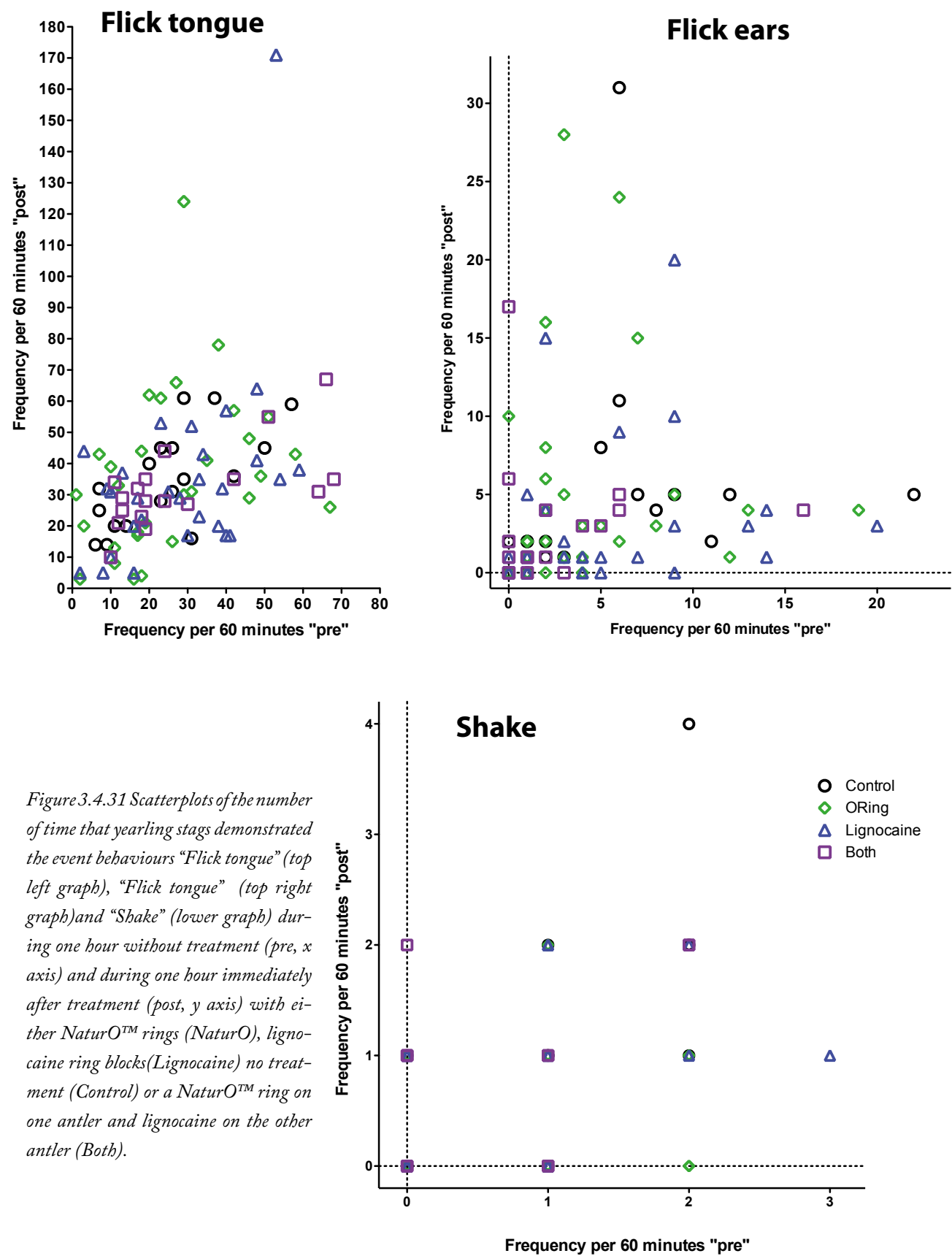


Figure 3.4.31 Scatterplots of the number of time that yearling stags demonstrated the event behaviours “Flick tongue” (top left graph), “Flick tongue” (top right graph) and “Shake” (lower graph) during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO™ rings (NaturO), lignocaine ring blocks (Lignocaine) no treatment (Control) or a NaturO™ ring on one antler and lignocaine on the other antler (Both).

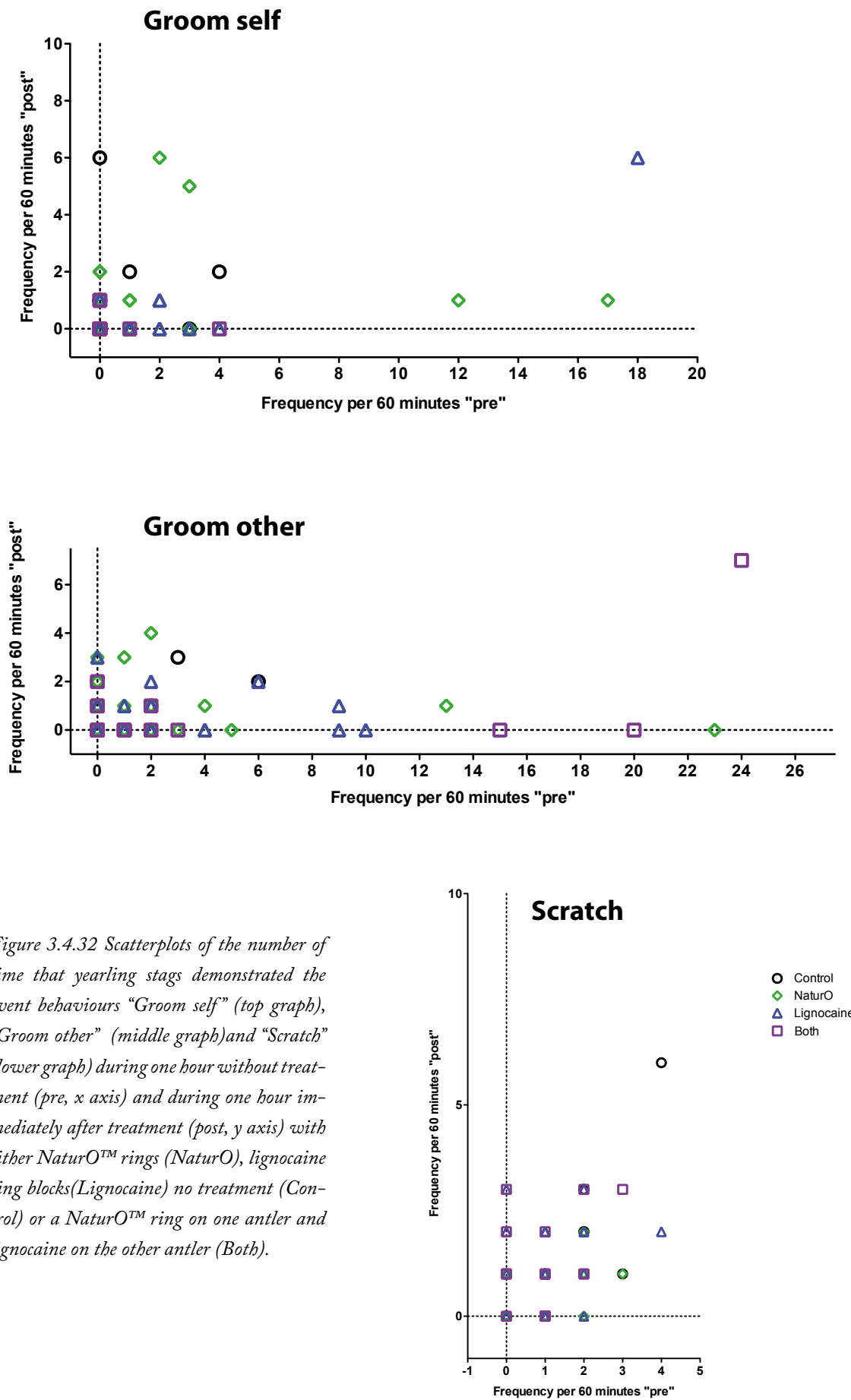


Figure 3.4.32 Scatterplots of the number of time that yearling stags demonstrated the event behaviours “Groom self” (top graph), “Groom other” (middle graph) and “Scratch” (lower graph) during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO™ rings (NaturO), lignocaine ring blocks (Lignocaine) no treatment (Control) or a NaturO™ ring on one antler and lignocaine on the other antler (Both).

Table 3.4.9 Correlation analyses, goodness of fit (Spearman R) and p-value for test for significant difference from zero for the number of times that deer demonstrated event behaviours without (pre) versus with (post) treatment with NaturO™ rings, lignocaine ring blocks or control. or both (NaturO on one antler, Lignocaine on other antler).

Where correlations were significant they are annotated below with “Sig” and where not significant with “Not sig”

Behaviour	Overall	Control	NaturO	Lignocaine	Both
Look up					
Spearman R	0.09392	0.3593	-0.04402	0.1866	-0.04292
p-value	0.3679	0.1431	0.8174	0.3325	0.8701
	Not sig	Not sig	Not sig	Not sig	Not sig
Nose to ground					
Spearman R	0.5902	0.5623	0.5848	0.7269	0.5695
p-value	< 0.0001	0.0151	0.0007	"< 0.0001"	0.0213
	Sig	Sig	Sig	Sig	Sig
Lick the walls					
Pearson R ²	0.2446	0.1810	0.1825	0.2815	0.3902
p-value	0.0175	0.4722	0.3344	0.1390	0.1216
	Sig	Not sig	Not sig	Not sig	Not sig
Flick tongue					
Spearman R	0.5004	0.6513	0.4637	0.4294	0.6129
p-value	< 0.0001	0.0034	0.0099	0.0201	0.0068
	Sig	Sig	Sig	Sig	Sig
Flick ears					
Spearman R	0.4300	0.6736	0.2838	0.4793	0.2786
p-value	< 0.0001	0.0022	0.1285	0.0085	0.2630
	Sig	Sig	Not sig	Sig	Not sig
Shake					
Spearman R	0.3495	0.7012	0.2797	0.2659	0.2412
p-value	0.0005	0.0012	0.1343	0.1633	0.3349
	Sig	Sig	Not sig	Not sig	Not sig
Groom self					
Spearman R	0.2213	0.3440	0.3488	0.1782	-0.1248
p-value	0.0312	0.1622	0.0589	0.3551	0.6218
	Sig	Not sig	Not sig	Not sig	Not sig
Groom other					
Spearman R	0.1677	0.5463	0.1286	0.09634	-0.01242
p-value	0.1043	0.0190	0.4984	0.6191	0.9610
	Not sig	Sig	Not sig	Not sig	Not sig
Scratch					
Spearman R	0.1824	0.7570	0.3019	0.3216	0.1094
p-value	0.0769	0.0003	0.1049	0.0889	0.6657
	Not sig	Sig	Not sig	Not sig	Not sig

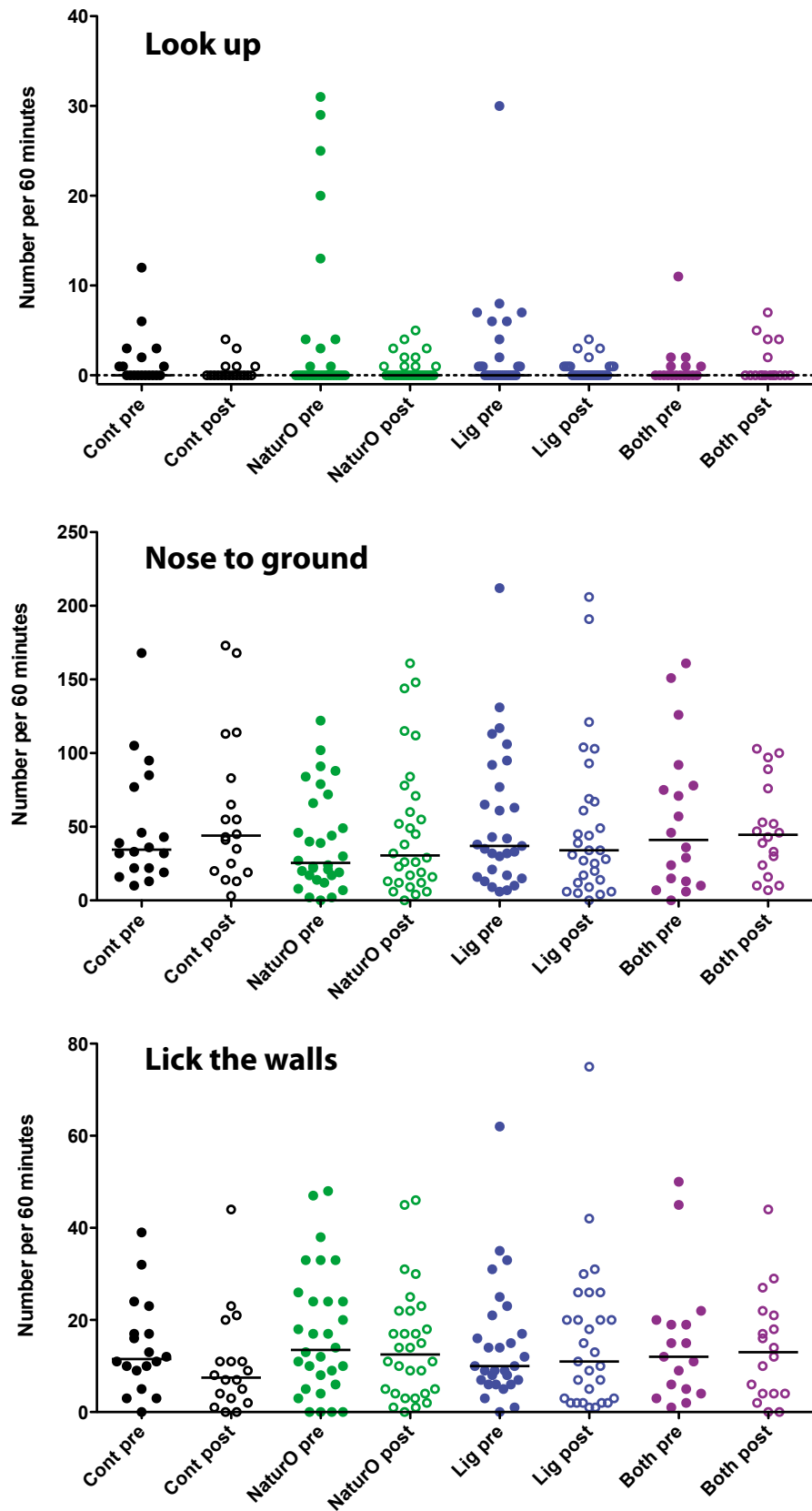


Figure 3.4.33 Individual value data plots of the number of times that one-year-old male deer demonstrated different event behaviours “Look up”, “Nose to ground” and “Lick the walls” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.

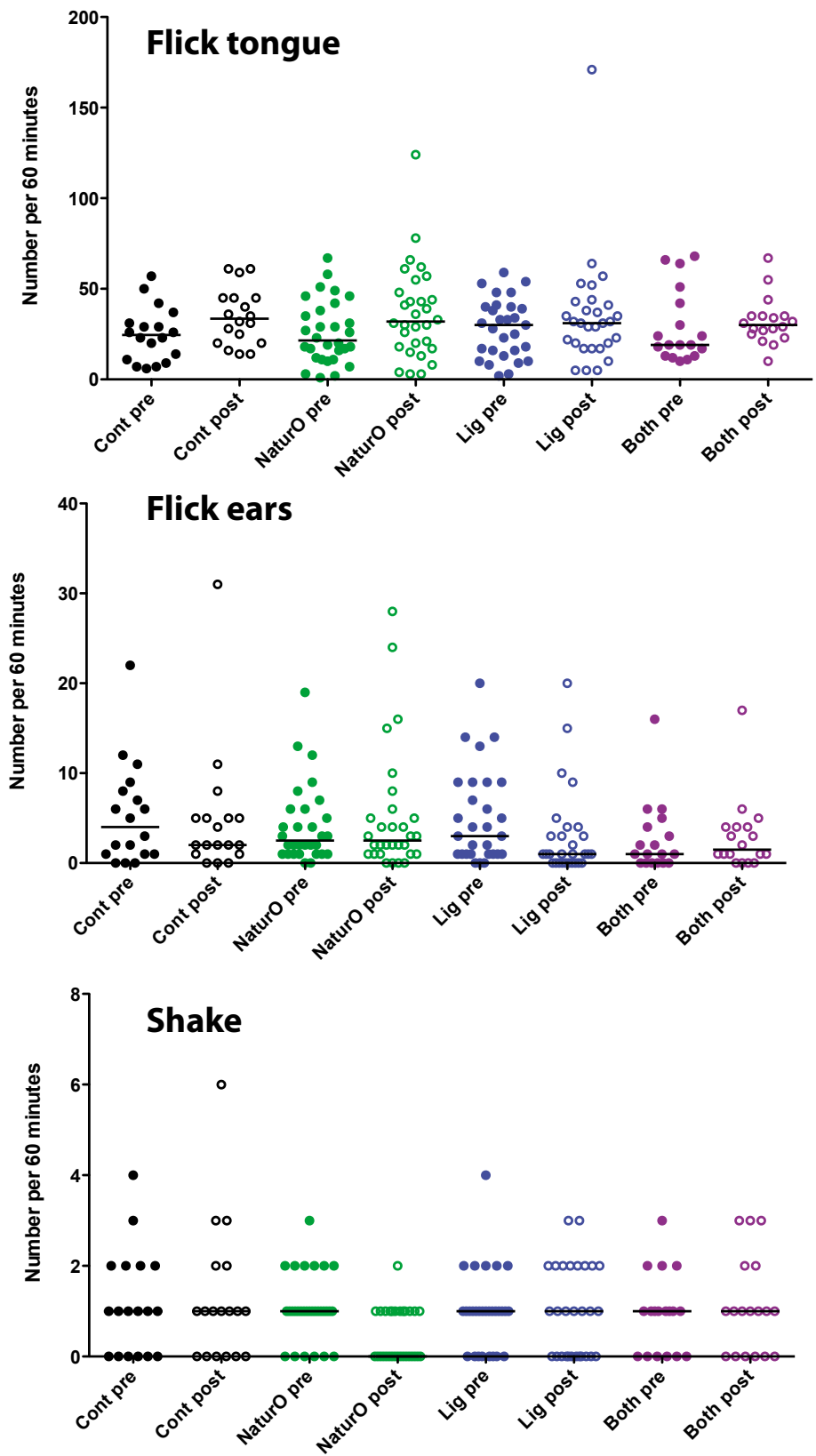


Figure 3.4.34 Individual value data plots of the number of times that one-year-old male deer demonstrated the different event behaviours: “Flick tongue”, “Flick ears” and “Shake” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.

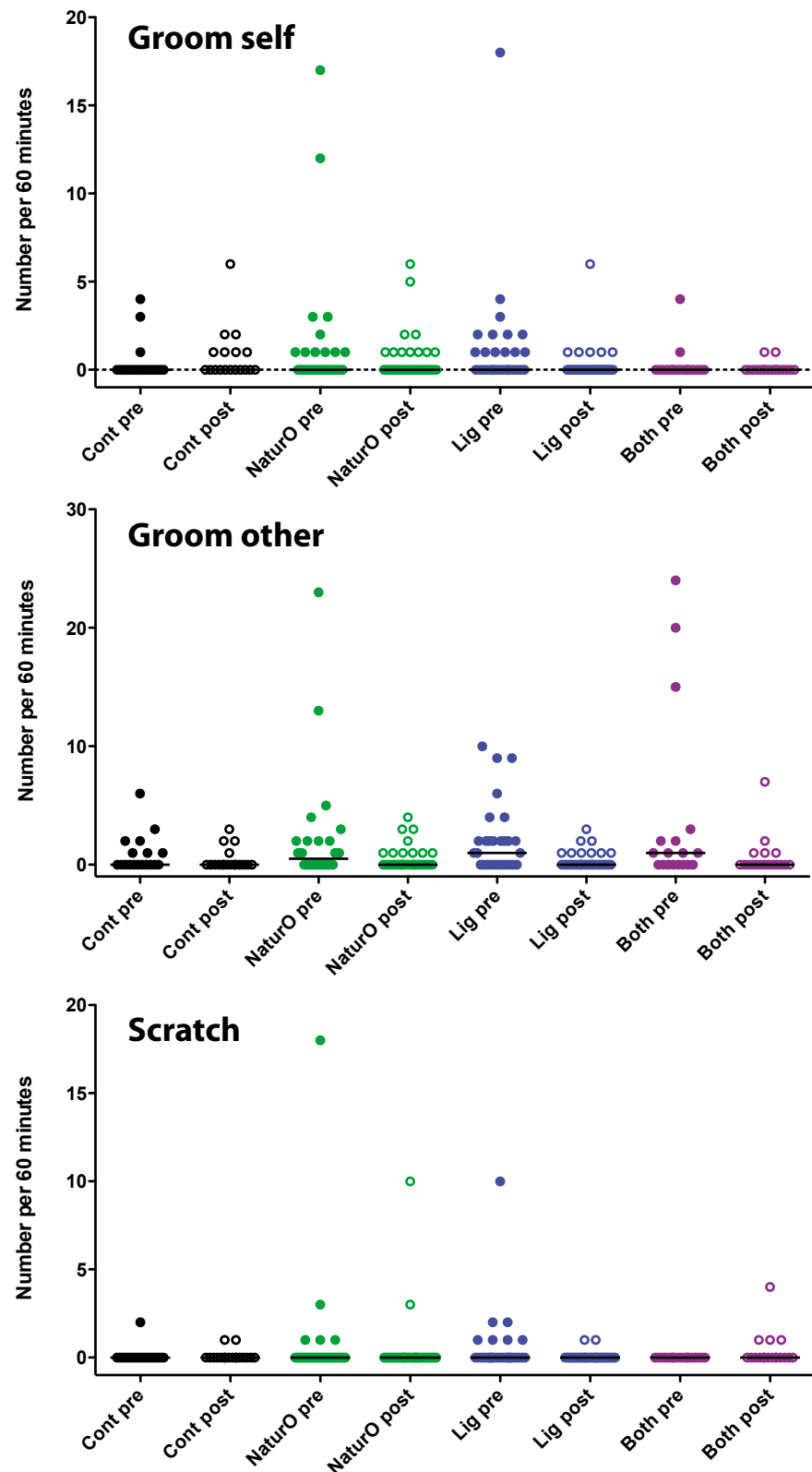


Figure 3.4.35 Individual value data plots of the number of times that one-year-old male deer demonstrated the different event behaviours “Groom self”, “Groom other” and “Scratch” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.

period than the “post” treatment period ($p=0.0038$).

Deer in the Both group demonstrated “Groom other” more often during the “pre” treatment period than the “post” treatment period ($p=0.043$).

Between treatment comparisons

Pre-treatment

Based on one-way ANOVA and post-ANOVA between group tests, there were no significant differences between any of the treatment groups for any of the non-specific event behaviours during the “pre” treatment period.

Post treatment

Based on one-way ANOVA and post-ANOVA between group tests, there were no significant differences between any of the treatment groups for any of the non-specific event behaviours during the “post” treatment period.

For the behaviour “Shake” Individual Mann-Whitney tests between treatment groups showed that deer in the *NaturO* group demonstrated the behaviour less frequently during the “post” treatment period than all other treatment groups.

Difference (Post - Pre)

Figure 3.4.36 shows the difference (post - pre treatment) in the frequency of each non-specific event behaviour for individual animals in each treatment group. There were no significant differences between any treatment groups in the mean difference(pre-post) for any of the behaviours.

Proportion of animals within groups that increased or decreased.

Table 3.4.10 shows the proportion of animals in each treatment group that doubled (“increase”) or halved (“decrease”) the frequency of each non-specific event behaviour during the “post” treatment period compared to the “pre” treatment period. The net change (%increase-%decrease) for each group is shown. When the net change was equal to $\pm 30\%$ or more it was considered of interest.

Look up

All groups except the Control group had some animals that showed “increase” and some that showed “decrease” in “Look up”.

The Control group had 7 out of 18 animals “decrease” and no animals “increase” resulting in a net change of -39% of animals.

NaturO and Lignocaine had 9 animals “decrease” but also had some animals “increase” so that the net change was not greater than 30%.

Nose to ground

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Nose to ground”.

The Lignocaine group had 12 animals “decrease” and 3 animals “increase” the frequency of “Nose to ground” resulting in a net change of -31%.

Lick the walls

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Lick the walls”.

In the *NaturO* group 15 animals “decreased” and 6 “increased” resulting in a net change of -30%.

In the Both group 9 animals “decreased” and 2 “increased” resulting in a net change of -39%.

Flick tongue

All treatment groups had some animals that “increased” the frequency of “Flick tongue”. The *NaturO* and Lignocaine groups also had animals that showed a “decrease” in flick tongue but the Control and Both groups did not.

In the *NaturO* group, 12 animals “increased” and 3 “decreased” resulting in a net change of +30%.

Flick ears

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Flick ears”

In the Lignocaine group 17 animals “decreased” and 5 animals “increased” resulting in a net change of -42%.

Shake

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Shake”

In the *NaturO* group 14 animals “decreased” and 1 animal “increased” resulting in a net change of -43%.

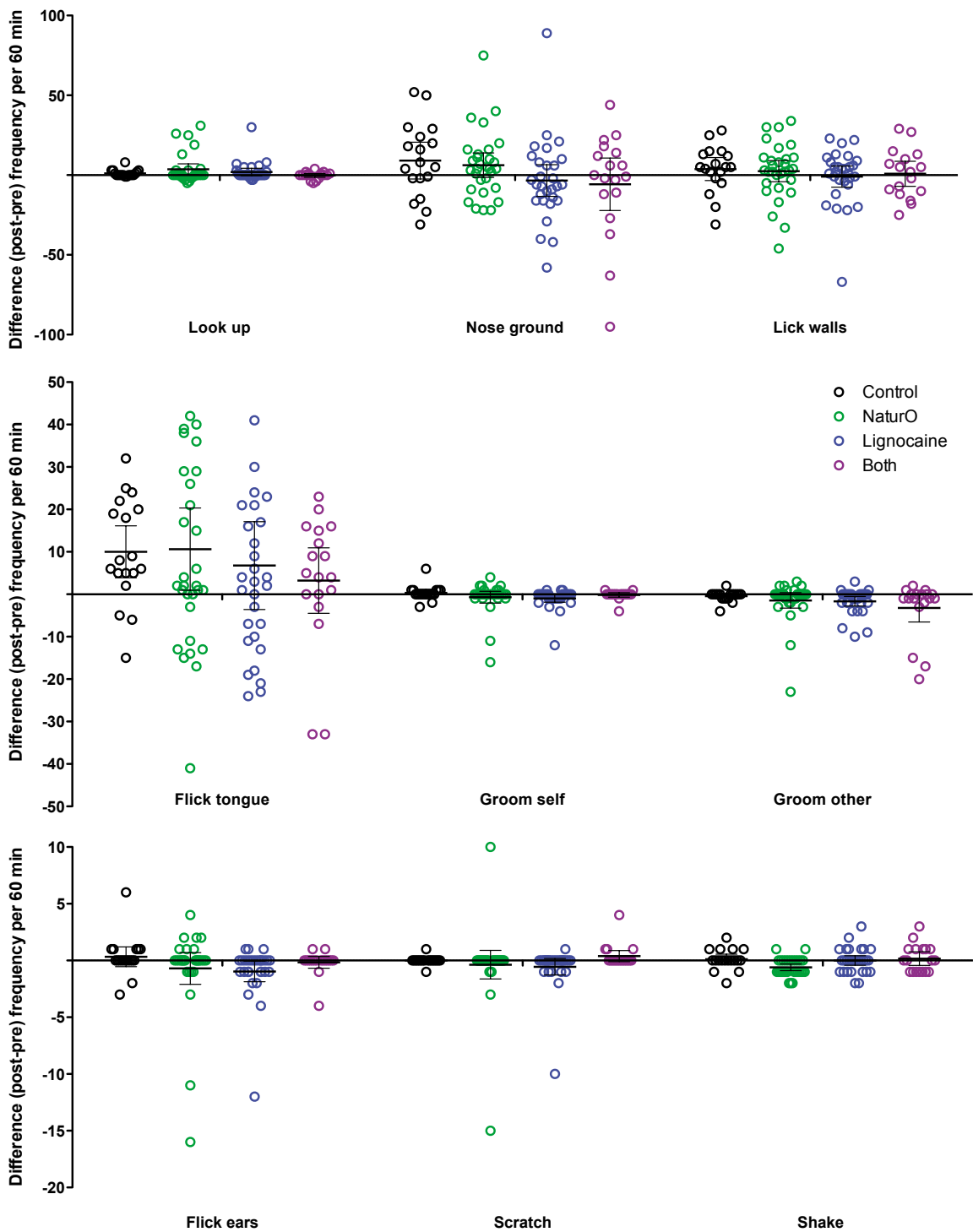


Figure 3.4.36 Individual data plot of the difference (“post” – “pre”) in the number of times that one-year-old male deer demonstrated different non-specific event behaviours during one hour of video recording after treatment with either NaturO™ rings, lignocaine ring blocks or no treatment around the antler pedicle compared to no treatment.

Scratch

All treatment groups had some animals that showed an “increase” and Control, *NaturO* and Lignocaine but not the Both group had some animals that showed a “decrease” in the frequency of “Scratch”.

No groups had a net change of $\geq \pm 30\%$.

Groom self

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Groom self”.

No groups had a net change of $\geq \pm 30\%$.

Groom other

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Groom other”.

In the Lignocaine group 13 animals “decreased” and 3 animals “increased” resulting in a net change of -34%.

In the Both group 9 animals “decreased” and 3 animals “increased” resulting in a net change of -33%.

Time series

Figure 3.4.37 shows the time series for “Look up”, “Nose to ground”, “Lick the walls”, “Flick tongue” and “Flick ears” during both the pre and post treatment periods in the top graph and the time series of “Groom other”, “Groom self”, “Scratch” and “Shake” during the “pre” and “post” treatment periods on the lower graph. Data shown are the total number of times that each behaviour was recorded across all deer during each 10 minute interval.

Look up

There were no significant differences between the “pre” and “post” treatment periods and there was no significant trend over time for the frequency of the behaviour “Look up”.

Nose to ground

There were significant differences between “pre” and “post” frequencies of “Nose the ground” during the last ten minute time period only. There were no significant trends over time.

Lick the Walls

There were no significant changes over time in the frequency of the behaviour “Lick the walls”

Table 3.4.10 Number of animals that showed a double “increase” or halve “decrease” in the frequency of the behaviours “Look up”, “Nose to ground”, “lick the walls”, “Flick tongue”, and “Flick ears” when post treatment recordings were compared to pre treatment recordings. % change indicates the overall proportion of deer in the group that showed positive changes.

Behaviour	Control	NaturO	Lignocaine	Both
Look up				
No change	11	15	16	13
Increase	0	6	4	3
Decrease	7	9	9	2
% change	-39%	-10%	-17%	+6%
Nose to ground				
No change	9	14	14	9
Increase	6	10	3	5
Decrease	3	6	12	4
% change	+16%	+13%	-31%	+6%
Lick the walls				
No change	6	9	16	7
Increase	4	6	5	2
Decrease	8	15	8	9
% change	-22%	-30%	-10%	-39%
Flick tongue				
No change	13	15	17	13
Increase	5	12	7	5
Decrease	0	3	5	0
% change	+28%	+30%	+7%	+28%
Flick ears				
No change	13	17	7	9
Increase	2	6	5	6
Decrease	3	7	17	3
% change	-6%	-3%	-42%	+17%

Table 3.4.10 Cont.

Behaviour	Control	NaturO	Lignocaine	Both
Shake				
No change	14	15	15	9
Increase	2	1	6	6
Decrease	2	14	8	3
% change	0%	-43%	-7%	+17%
Scratch				
No change	16	24	21	14
Increase	1	1	1	4
Decrease	1	5	7	0
% change	0%	-13%	-21%	+22%
Groom self				
No change	12	17	15	14
Increase	5	6	3	2
Decrease	1	7	11	2
% change	+22%	-3%	-28%	0%
Groom other				
No change	12	14	13	6
Increase	1	5	3	3
Decrease	5	11	13	9
% change	-22%	-20%	-34%	-33%

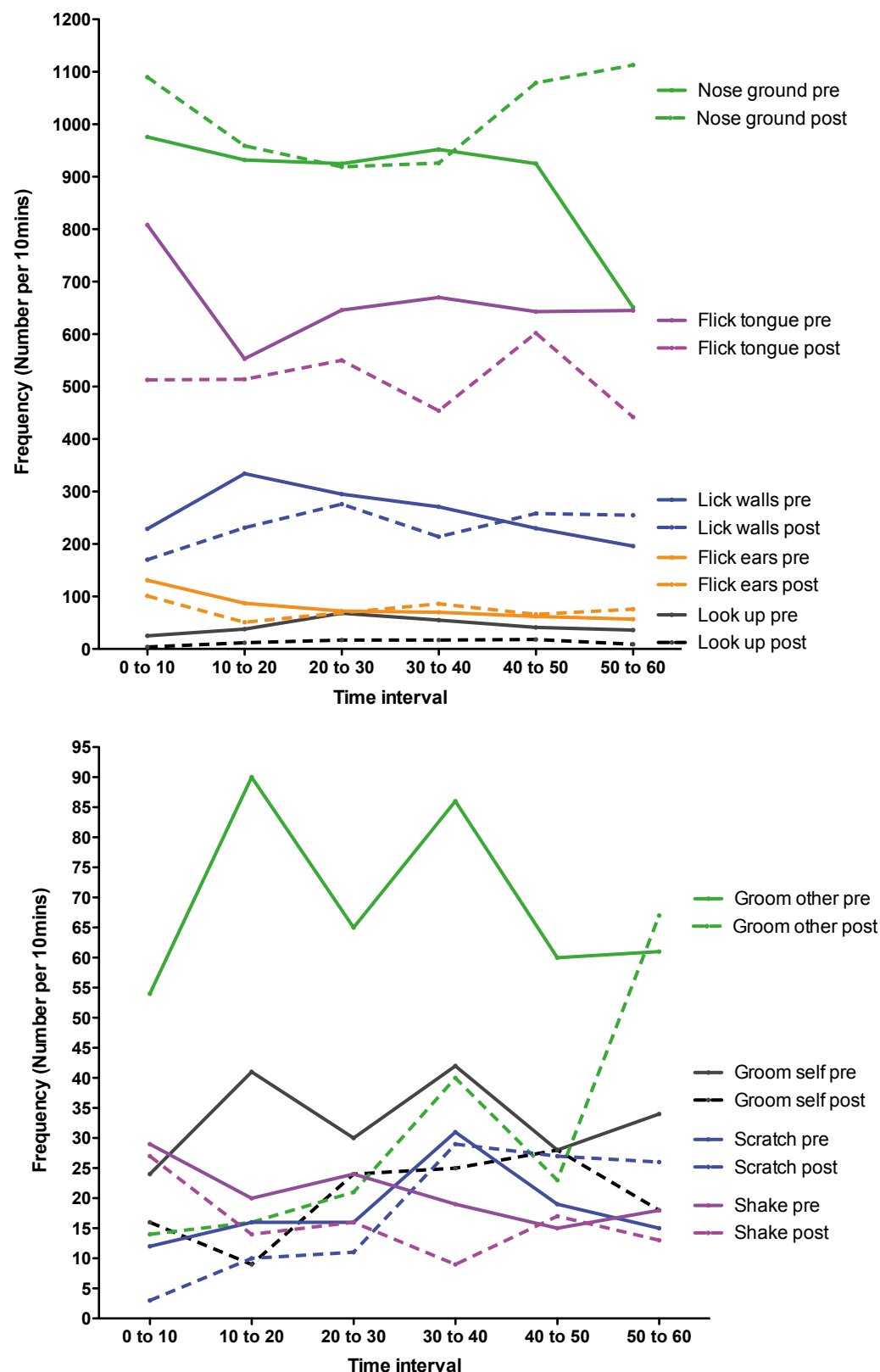


Figure 3.4.37 Time-series plot showing the mean number of time that one-year-old deer demonstrated different non-specific event behaviours during each 10 minute period of 60 minutes of video observation of deer in pens either without “pre” or following “post” treatment with NaturO™ rings, lignocaine ring blocks, no treatment or combinations of treatment. All treatment groups are combined.

during either the “pre” or “post” treatment periods.

Flick tongue

The median frequency of “Flick tongue” was highest during the first 10 minute period and this was consistent for all treatment groups during the “post” treatment period but not the “pre” treatment period. During the time after 10 minutes the frequency was variable and did not show a significant pattern although all groups except “Both” tended to have a higher frequency of “Tongue flick” during the “post” treatment periods than the “pre” treatment periods.

Flick ears

Overall, the frequency of “flick ears” was highest in the first 10 minutes after release into the observation pens and became variable thereafter. This was true for all groups except “Control” during the “post” treatment and “NaturO” during the “post” treatment recordings.

Groom self

There was no pattern over time of the frequency of the behaviour “Groom self”.

Groom other

There was no clear pattern over time of the frequency of “Groom other”.

Shake

Overall, the frequency of “Shake” was highest in the first 10 minutes after release into the observation pens and became variable thereafter.

Specific Head Related Behaviours

Data Distribution

Rotate left

A total of 22/95 (23%) and 36/95 (38%) animals demonstrated the behaviour “Rotate left” during the “pre” and “post” treatment periods respectively. This difference was statistically significant ($p=0.02$). The mean number of times that deer demonstrated “Rotate Left” was 0.8 and 3.97 for “pre” and “post” treatment recordings respectively. The range was 0 to 31 and the data were not normally distributed and were skewed to the right. The median “pre” and “post” treatment values were significantly different (Wilcoxin p -value =0.0002). The distribution of the frequency of the behaviour “Rotate left” for each deer is shown in Figure 3.4.38 (top graph) with “pre” and “post” shown separately.

Rotate right

A total of 24/95 (25%) and 39/95(41%) animals demonstrated the behaviour “Rotate right” during the “pre” and “post” treatment periods respectively. This difference was statistically significant ($p=0.018$). The mean number of times that deer demonstrated “Rotate right” was 1.13 and 4.65 for “pre” and “post” treatment recordings respectively. The range was 0 to 86 and the data were not normally distributed and were skewed to the right. The median “pre” and “post” treatment values were significantly different (Wilcoxin p -value =0.0014). The distribution of the frequency of the behaviour “Rotate right” for each deer is shown in Figure 3.4.38 (middle graph) with “pre” and “post” shown separately.

Turn head 360

A total of 4/95 (4%) and 15/95 (16%) animals demonstrated the behaviour “Turn head 360” during the “pre” and “post” treatment periods respectively. This difference was statistically significant ($p=0.007$). The mean number of times that deer demonstrated “Turn head 360” was 0.13 and 0.99 for “pre” and “post” treatment recordings respectively. The range was 0 to 33 and the data were not normally distributed and were skewed to the right. The median “pre” and “post” treatment values were significantly different (Wilcoxin p -value =0.0076). The distribution of the frequency of the behaviour “Turn head 360” for each deer is shown in Figure 3.4.38 (lower graph) with “pre” and “post” shown separately.

Arch head down

A total of 44/95 (46%) and 66/95 (69%) animals demonstrated the behaviour “Arch head down” during the “pre” and “post” treatment periods respectively. This difference was statistically significant ($p=0.0009$). The mean number of times that deer demonstrated “Arch head down” was 4.36 and 7.40 for “pre” and “post” treatment recordings respectively. The range was 0 to 88 and the data were not normally distributed and were skewed to the right. The median “pre” and “post” treatment values were significantly different (Wilcoxin p -value =0.040). The distribution of the frequency of the behaviour “Arch head down” for each deer is shown in Figure 3.4.39 (top graph) with “pre” and “post” shown separately.

Arch head up

A total of 37/95(39%) and 51/95(54%) animals demonstrated the behaviour “Arch head up” during the “pre” and “post” treatment periods respectively. This difference was statistically significant ($p=0.039$). The mean number of times that deer demonstrated “Arch head up” was 1.58 and 3.82 for “pre” and “post” treatment recordings respectively. The range was 0 to 73 and the data were not normally distributed and were skewed to the right. The median “pre” and “post” treatment values were significantly different (Wilcoxin p -value =0.028). The distribution of the frequency of the behaviour “Arch head up” for each deer is shown in Figure 3.4.39 (middle graph) with “pre” and “post” shown separately.

Press Head

A total of 39/95 (41%) and 44/95 (46%) animals demonstrated the behaviour “Press head” during the “pre” and “post” treatment periods respectively. This difference was not statistically significant ($p=0.47$). The mean number of times that deer demonstrated “Press head” was 2.7 and 3.1 for “pre” and “post” treatment recordings respectively. The range was 0 to 46 and the data were not normally distributed and were skewed to the right. The median “pre” and “post” treatment values were not significantly different (Wilcoxin p -value =0.4025). The distribution of the frequency of the behaviour “Press head” for each deer is shown in Figure 3.4.39 (lower graph) with “pre” and “post” shown separately.

“Pre” vs “post” correlation analysis

Figures 3.4.40 and 3.4.41 shows scatterplots of the number of times that individual deer demonstrated different specific head related event behaviours during the pre-treatment recording time (x-axis) and post-treatment recording time (y-axis). Treatment group for each animal is indicated by a different coloured symbol. Table 3.4.11 shows the goodness of fit and p -values for significant correlations for pre vs post treatment recordings for each behaviour. As all data were not normally distributed, Spearman R values are shown.

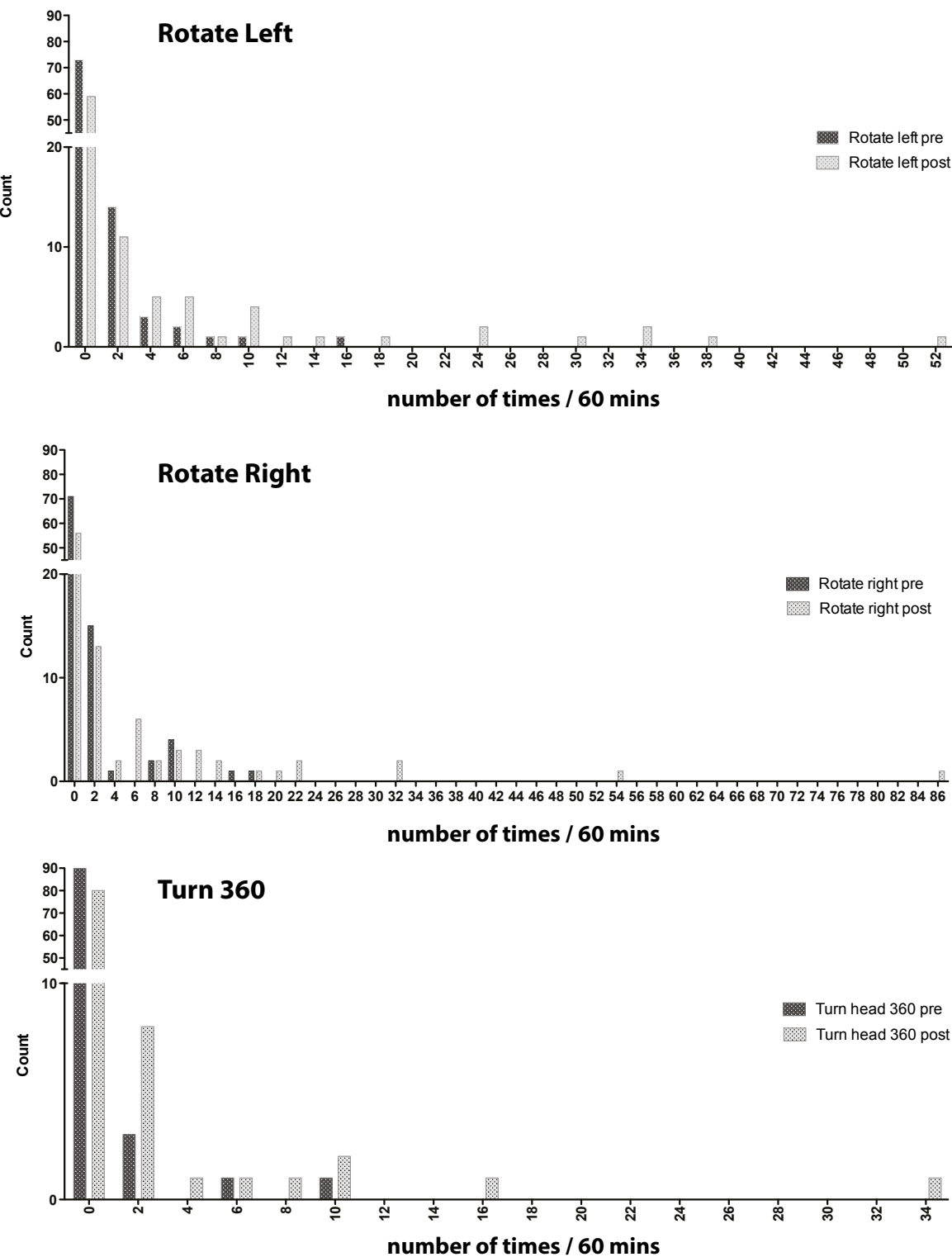


Figure 3.4.38 Histograms of the frequency distribution of the number of times one-year-old male deer demonstrated different specific head related event behaviours “Rotate left” (top graph), “Rotate right” (middle graph) and “Turn head 360” (lower graph) during 60 minutes of video observation in a pen. “Pre” and “post” treatment values shown separately. All treatment groups are combined. “Count” = number of deer.

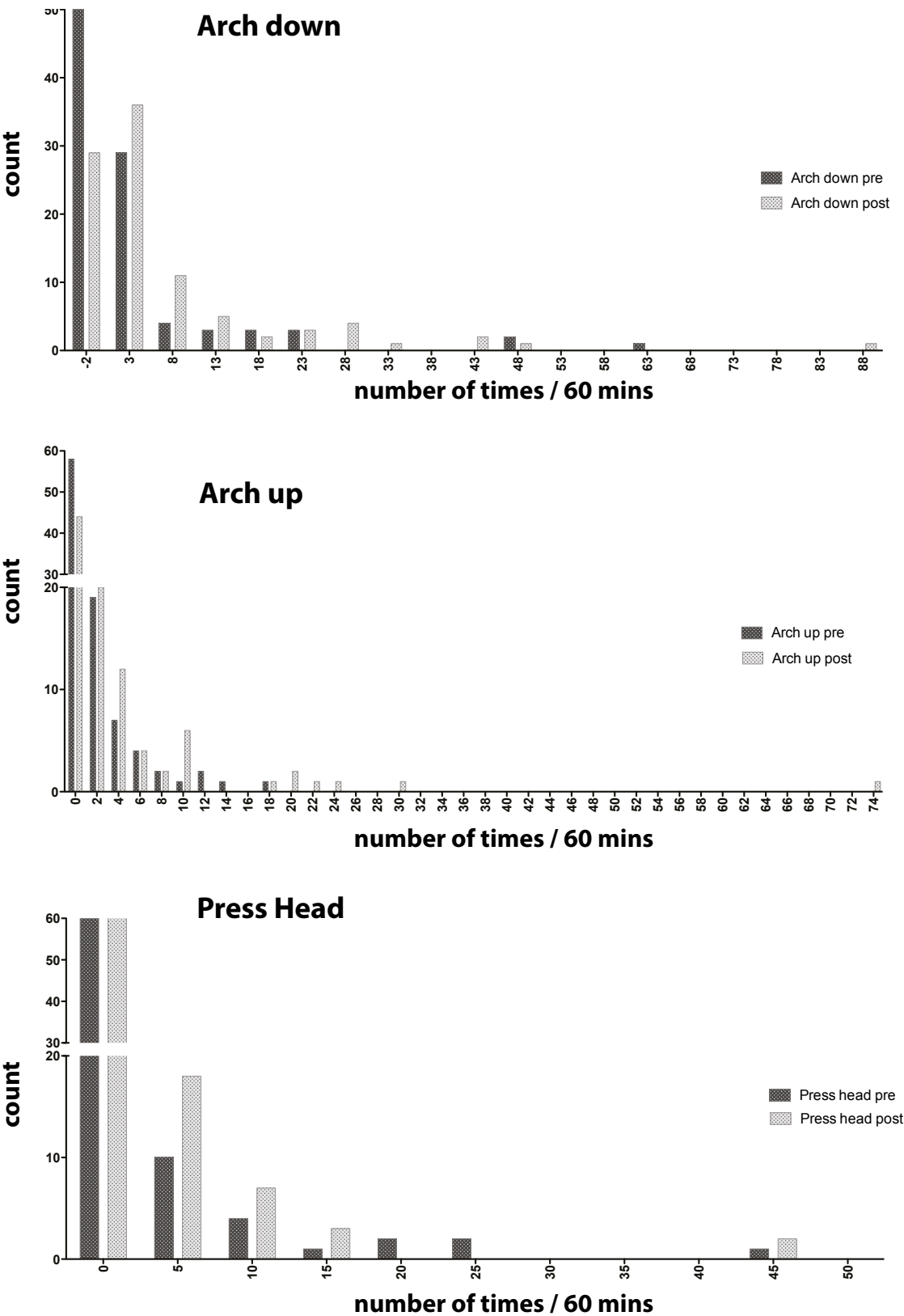


Figure 3.4.39 Histograms of the frequency distribution of the number of times one-year-old male deer demonstrated different specific head related event behaviours “Arch down” (top graph), “Arch up” (middle graph) and “Press head” (lower graph) during 60 minutes of video observation in a pen. “Pre” and “post” treatment values shown separately. All treatment groups are combined. “Count” = number of deer.

Rotate left

There were no significant correlations within any of the treatment groups or overall for the “pre” versus “post” frequency of “Rotate left”.

Rotate right

The correlation between the “pre” and “post” frequencies for “Rotate right” was not significantly different from zero overall or within any treatment except Control.

Turn head 360

There were no significant correlations within any of the treatment groups or overall for the “pre” versus “post” frequency of “Turn head 360°”. In the Control and Lignocaine groups data could not be analysed because all of the values during the “pre” treatment period were zero for the Control group and all of the values during the “post” treatment period were zero for the Lignocaine group.

Arch head down

The correlation between the “pre” and “post” frequencies for “Arch head down” was significantly different from zero overall. For individual treatment groups there was a significant correlation in the Control group only.

Arch head up

The correlation between the “pre” and “post” frequencies for “Arch head up” was not significantly different from zero overall or within any treatment except Control.

Press Head

There were no significant correlations within any of the treatment groups or overall for the “pre” versus “post” frequency of “Press head”.

Comparisons within and between treatment groups

Figures 3.4.42 and 3.4.43 show individual animal data plots of the frequency of specific head related event behaviours during the “pre” and “post” treatment periods for each treatment group. ANOVA was used to test for significant difference between the medians across all groups and individual Wilcoxin matched pairs sign ranked tests were used to compare medians within treatments.

“Pre” vs “post” comparison within groups

Rotate left

Figure 3.4.42 (top graph) shows the number of times deer in each treatment group “Rotated right” during the “pre” and “post” treatment periods for each treatment group. Wilcoxin Signed ranked test found the following:

In the *NaturO* group, “Rotate left” was demonstrated more often during the “post” treatment period than the “pre” treatment period. $p < 0.0001$

In the Both group, “Rotate left” was demonstrated more often during the “post” treatment period than the “pre” treatment period $p = 0.032$

Rotate right

Figure 3.4.42 (middle graph) shows the number of times deer in each treatment group “Rotated right” during the “pre” and “post” treatment periods for each treatment group. Wilcoxin Signed ranked test found the following:

In the *NaturO* group, “Rotate right” was demonstrated more often during the “post” treatment period than the “pre” treatment period. $p = 0.0004$

In the Both group, “Rotate right” was demonstrated more often during the “post” treatment period than the “pre” treatment period $p = 0.046$

Turn head 360

Figure 3.4.42 (lower graph) shows the number of times deer in each treatment group “Turned head 360” during the “pre” and “post” treatment periods for each treatment group. Wilcoxin Signed ranked test found the following:

In the *NaturO* group, “Turn head 360” was demonstrated more often during the “post” treatment period than the “pre” treatment period. $p = 0.0001$

In the Both group, “Turn head 360” was demonstrated more often during the “post” treatment

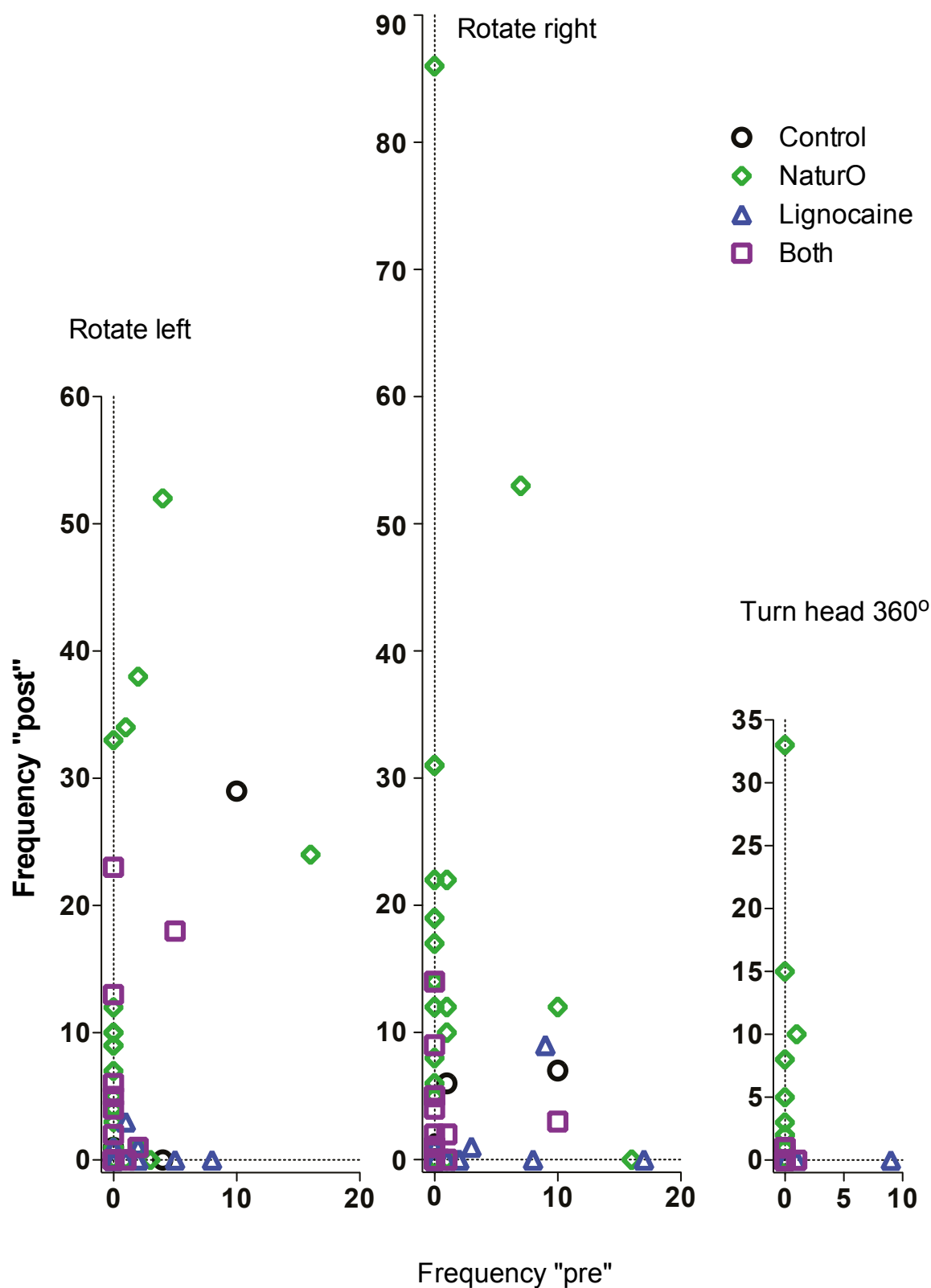


Figure 3.4.40 Scatterplots of the number of time that yearling stags demonstrated the specific head related event behaviours “Rotate left” (left graph), “Rotate right” (middle graph) and “Turn head 360°” (right graph) during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO™ rings (NaturO), lignocaine ring blocks (Lignocaine) no treatment (Control) or a NaturO™ ring on one antler and lignocaine on the other antler (Both). All axis are the same scale

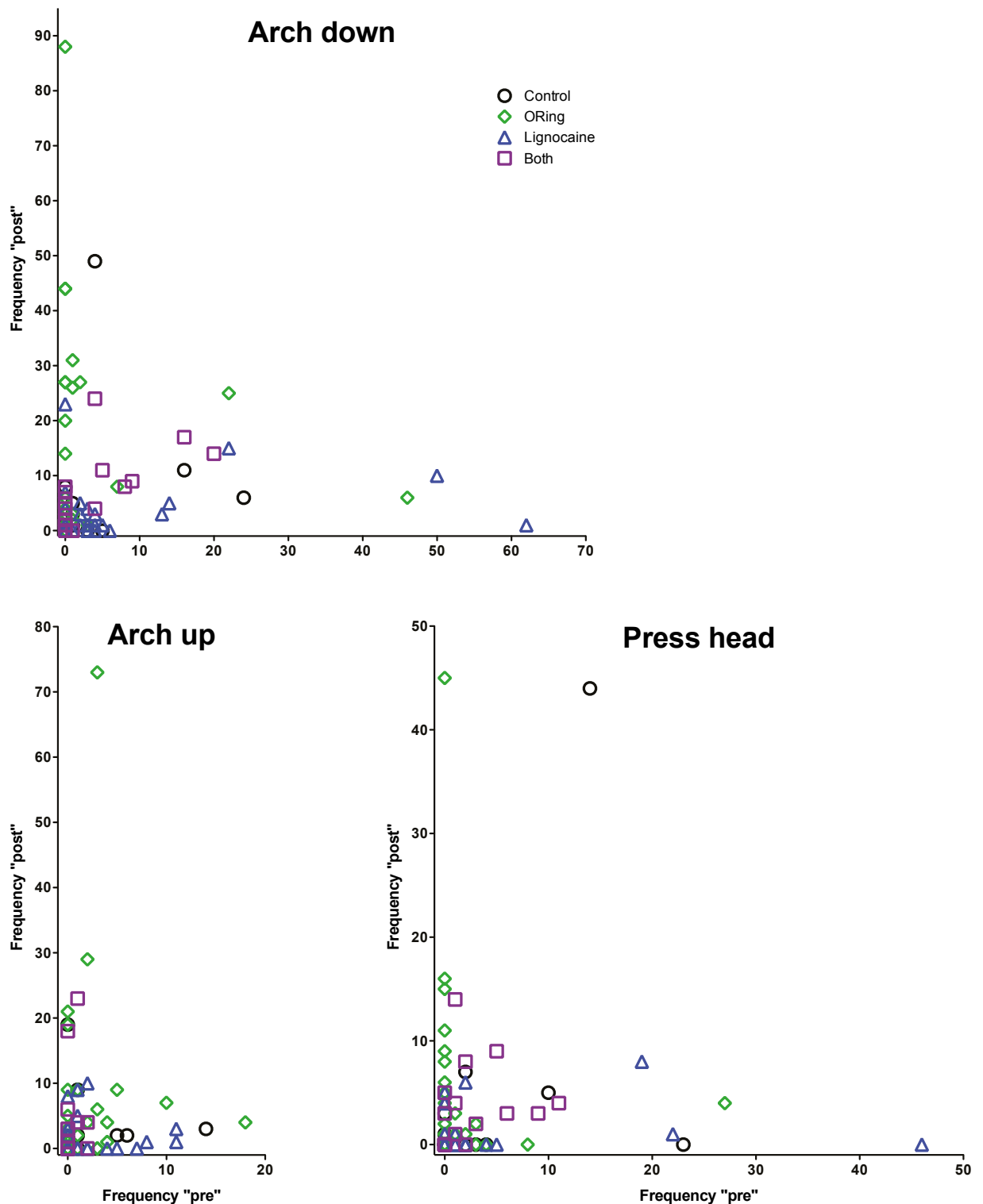


Figure 3.4.41 Scatterplots of the number of time that yearling stags demonstrated the specific head related event behaviours "Arch down" (top graph), "Arch up" (lower left graph) and "Press head" (lower right graph) during one hour without treatment (pre, x axis) and during one hour immediately after treatment (post, y axis) with either NaturO™ rings (NaturO), lignocaine ring blocks (Lignocaine) no treatment (Control) or a NaturO™ ring on one antler and lignocaine on the other antler (Both).

Table 3.4.11 Correlation analyses, goodness of fit (Spearman R) and p-value for test for significant difference from zero for the number of times that deer demonstrated specific head related event behaviours without (pre) versus with (post) treatment with NaturO™ rings, lignocaine ring blocks or control. or both (NaturO on one antler, Lignocaine on other antler).

Where correlations were significant they are annotated below with “Sig” and where not significant with “Not sig”

Behaviour	Overall	Control	<i>NaturO</i>	Lignocaine	Both
Rotate left					
Spearman R	0.1473	0.3956	0.2623	0.09807	0.09031
p-value	0.1543	0.1042	0.1614	0.6128	0.7216
	Not sig	Not sig	Not sig	Not sig	Not sig
Rotate right					
Spearman R	0.0985	0.5583	0.1270	0.1644	-0.1169
p-value	0.3422	0.0160	0.5037	0.3941	0.6441
	Not sig	Sig	Not sig	Not sig	Not sig
Turn head 360°					
Spearman R	0.1801	Insufficient	0.3197	Insufficient	-0.1157
p-value	0.0807	data	0.0850	data	0.6583
	Not Sig		Not sig		Not sig
Arch head down					
Spearman R	0.2515	0.4937	0.2417	0.2849	0.6835
p-value	< 0.0139	0.0373	0.1981	0.1342	0.0025
	Sig	Sig	Not sig	Not sig	Not sig
Arch head up					
Spearman R	0.0525	0.6425	0.2632	0.1561	0.3220
p-value	0.6136	0.0040	0.1600	0.4187	0.1926
	Not Sig	Sig	Not sig	Not sig	Not sig
Press head					
Spearman R	0.1122	0.3523	-0.1771	0.08260	0.4723
p-value	0.2792	0.1516	0.3490	0.6701	0.0556
	Not Sig	Not sig	Not sig	Not sig	Not sig

period than the “pre” treatment period $p=0.034$

Arch head down

Figure 3.4.43 (Top graph) shows the number of times deer in each treatment group “Arched head down” during the “pre” and “post” treatment periods for each treatment group. Wilcoxin Signed ranked test found the following:

In the *NaturO* group, “Arch head down” was demonstrated more often during the “post” treatment period than the “pre” treatment period. $p=0.0007$

In the Both group, “Arch head down” was demonstrated more often during the “post” treatment period than the “pre” treatment period $p=0.022$

Arch head up

Figure 3.4.43 (middle graph) shows the number of times deer in each treatment group “Arched head up” during the “pre” and “post” treatment periods for each treatment group. Wilcoxin Signed ranked test found the following:

In the *NaturO* group, “Arch head up” was demonstrated more often during the “post” treatment period than the “pre” treatment period. $p=0.025$

In the Both group, “Arch head up” was demonstrated more often during the “post” treatment period than the “pre” treatment period $p=0.012$

Press Head

Figure 3.4.43 (middle graph) shows the number of times deer in each treatment group demonstrated “Press head” during the “pre” and “post” treatment periods for each treatment group.

Wilcoxin Signed ranked test found no significant differences within any of the treatment groups.

“Pre”, “post” and “diff” comparisons between treatment groups

Pre-treatment

Across all behaviours and treatment groups the only significant differences during the pre-treatment recording were:

Animals in the Lignocaine group demonstrated “Arch down” more often than deer in the *NaturO* group ($p=0.0063$)

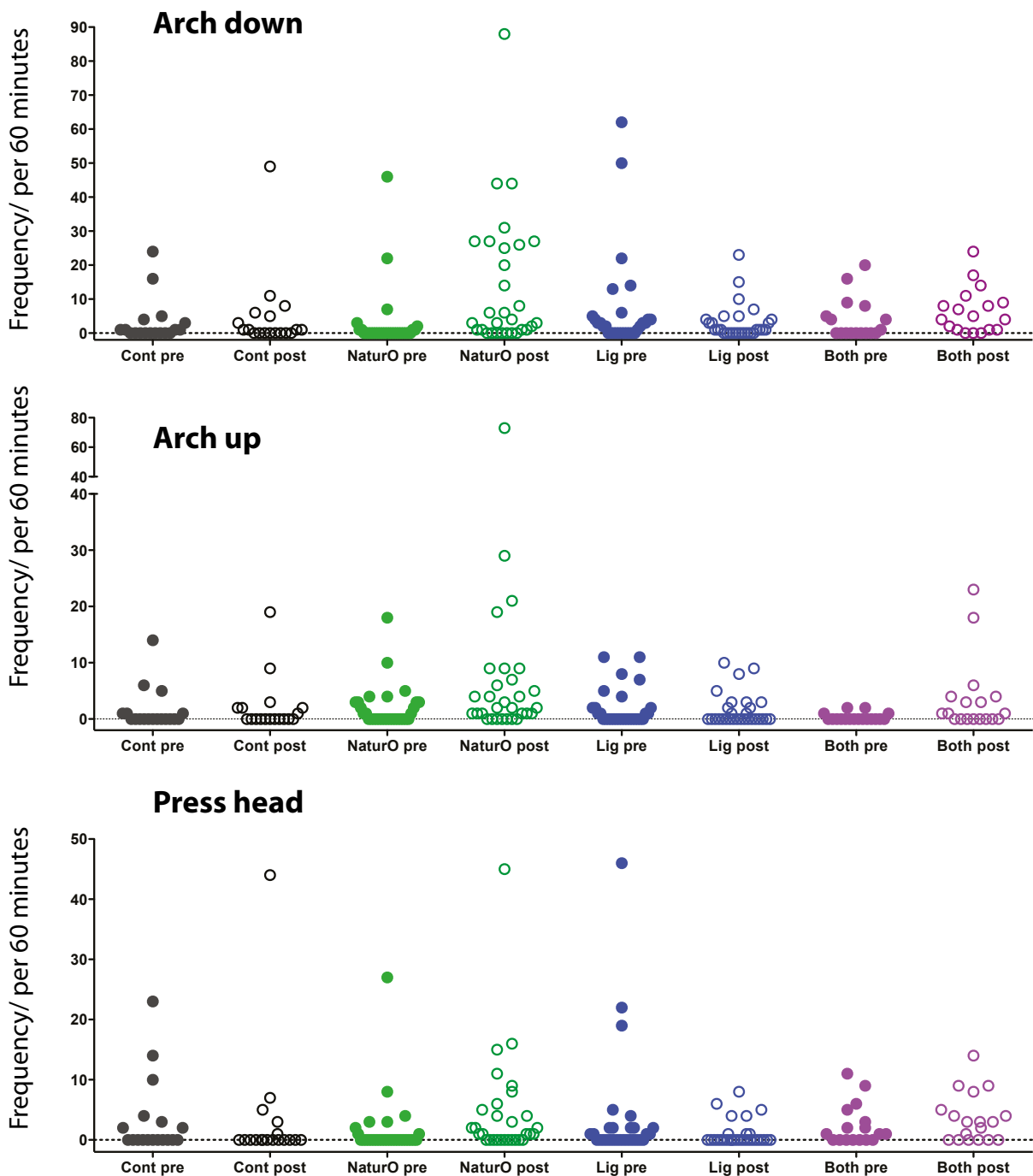


Figure 3.4.43 Individual value data plots of the number of times that one-year-old male deer demonstrated the different head position behaviours “Arch down”, “Arch up” and “Press head” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, both or no treatment on the antler pedicle.

Animals in the Lignocaine group demonstrated “Head Twist” more often than deer in the *NaturO* group ($p=0.049$)

Post treatment

Rotate left

NaturO “Rotated left” more often than Control ($p=0.0002$)

NaturO “Rotated left” more often than Lignocaine ($p<0.0001$)

Both “Rotated left” more often than Control ($p=0.034$)

Both “Rotated left” more often than Lignocaine ($p=0.016$)

Rotate right

NaturO “Rotated right” more often than Control ($p=0.0005$)

NaturO “Rotated right” more often than Lignocaine ($p<0.0001$)

Both “Rotated right” more often than Control ($p=0.014$)

Both “Rotated right” more often than Lignocaine ($p=0.0011$)

Turn head 360

NaturO “Turned head 360” more often than Control ($p=0.0002$)

NaturO “Turned head 360” more often than Lignocaine ($p<0.0001$)

Both “Turned head 360” more often than Control ($p=0.034$)

Both “Turned head 360” more often than Lignocaine ($p=0.016$)

Arch head down

NaturO “Arched head down” more often than Control ($p=0.045$)

NaturO “Arched head down” more often than Lignocaine ($p=0.019$)

Both “Arched head down” more often than Lignocaine ($p=0.027$)

Arch head up

NaturO “Arched head up” more often than Control ($p=0.016$)

NaturO “Arched head up” more often than Lignocaine ($p=.0047$)

Press Head

NaturO “Press head” more often than Control ($p=0.014$)

NaturO “Press head” more often than Lignocaine ($p<0.006$)

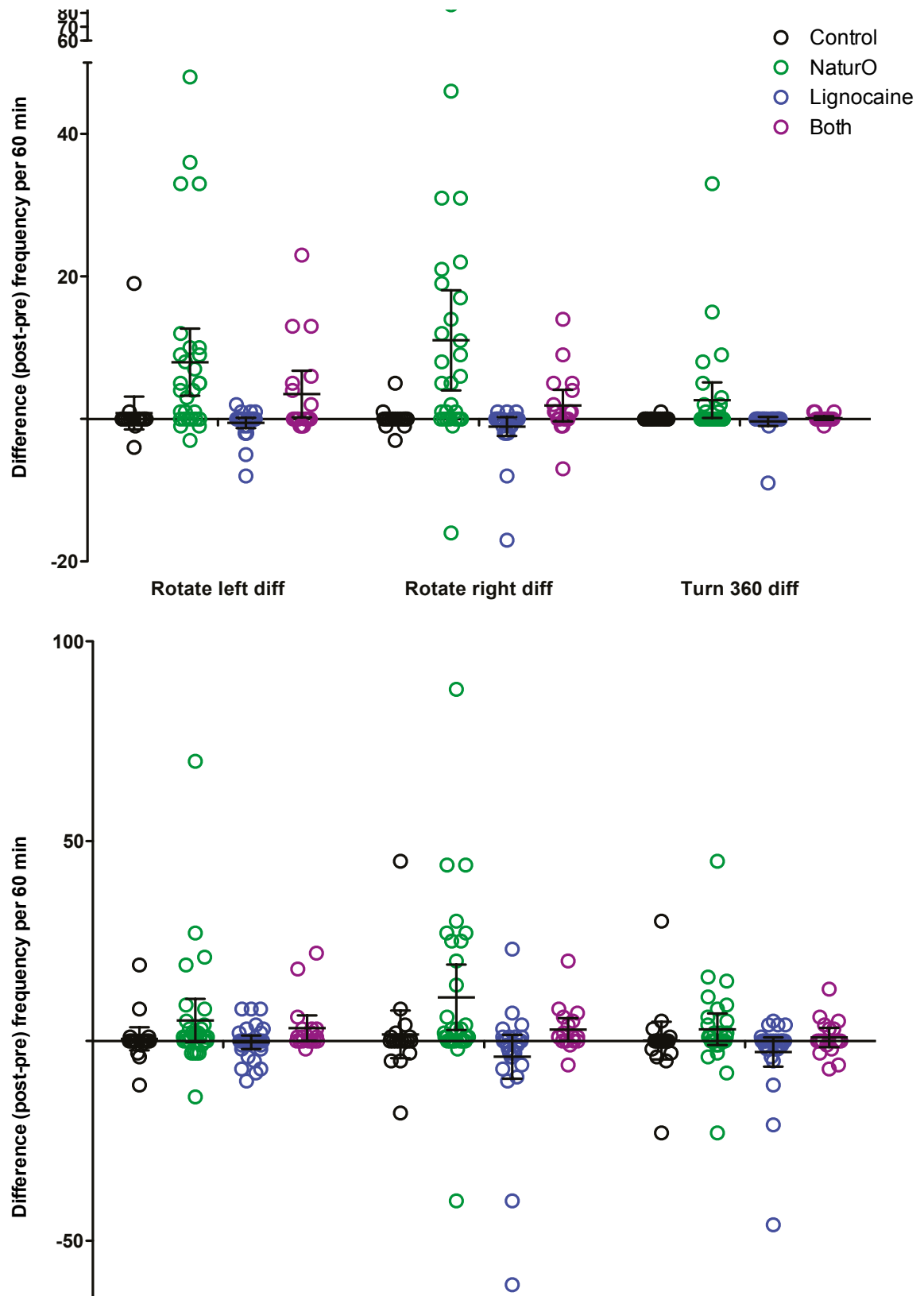


Figure 3.4.44 Individual data plot of the difference ("post" - "pre") in the number of times that one-year-old male deer demonstrated different specific head related event behaviours during one hour of video recording after treatment with either No treatment (Control), NaturOTM rings, lignocaine ring blocks or a NaturOTM ring on one antler and lignocaine ring block on the other (both).

Both “Press head” more often than Control ($p=0.037$)

Both “Press head” more often than Lignocaine ($p=0.0064$)

Difference (Post - Pre)

Figure 3.4.44 shows the difference (post - pre treatment) in the frequency of each specific head related event behaviour for individual animals in each treatment group. There were no significant differences between any treatment groups in the mean difference(pre-post) for any of the behaviours.

Proportion of animals within groups that “increased” or “decreased”

Table 3.4.12 shows the number of deer that doubled (“increase”) or halved (“decrease”) or had “no change” in the frequency of specific head related behaviours between “pre” and “post” treatment recordings for each treatment group. Also shown is the net proportion of animals that doubled or halved the frequency of each behaviour in each treatment group.

Rotate left

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Rotate left”.

In the *NaturO* group, 20/30 animals “increased” and 2/30 “decreased” resulting in a net change of +60%.

Rotate right

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Rotate right”.

In the *NaturO* group 18/30 animals “increased” and 2/30 “decreased” resulting in a net change of +53%.

In the Both group, 10/18 animals “increased” and 3/18 “decreased” resulting in a net change of +39%

Turn head 360°

In the Control group, no animals either “increased” or “decreased” the frequency of “Turn head 360°”.

Table 3.4.12 Number of animals that showed an 50% “increase” or “decrease” in the frequency of the behaviour when post treatment recordings were compared to pre treatment recordings. % change indicates the overall proportion of deer in the group that showed positive changes.

Behaviour	Control	NaturO	Lignocaine	Both
N	18	30	29	18
Rotate left				
No change	16 (89%)	8 (27%)	21 (72%)	9 (50%)
Increase	1 (6%)	20 (67%)	3 (10%)	7 (39%)
Decrease	1 (6%)	2 (7%)	5 (17%)	2 (11%)
% change	0%	+60%	-7%	+28%
Rotate right				
No change	14 (78%)	10 (33%)	21 (72%)	5 (28%)
Increase	2 (11%)	18(60%)	2 (7%)	10 (56%)
Decrease	2 (11%)	2 (7%)	6 (21%)	3 (17%)
% change	0%	+53%	-14%	+39%
Turn head 360				
No change	18 (100%)	21 (70%)	28 (97%)	13 (72%)
Increase	0	9 (30%)	0	4(22%)
Decrease	0	0	1 (3%)	1 (6%)
% change	0%	+30%	-3%	+17%
Arch down				
No change	11 (61%)	10 (33%)	11 (38%)	6 (33%)
Increase	6 (33%)	19 (63%)	7 (24%)	10 (56%)
Decrease	1 (6%)	1 (3%)	11 (38%)	2 (11%)
% change	+28%	+60%	-14%	+44%
Arch up				
No change	13 (72%)	9 (30%)	14 (48%)	8 (44%)
Increase	4 (22%)	16 (53%)	5 (17%)	8 (44%)
Decrease	1 (6%)	5 (17%)	10 (34%)	2 (11%)
% change	+17%	+37%	-17%	+33%
Press head				
No change	11 (61%)	16 (53%)	17 (59%)	8 (44%)
Increase	3 (17%)	11 (37%)	3 (10%)	7 (39%)
Decrease	4 (22%)	1 (3%)	9 (31%)	2 (11%)
% change	-6%	+40%	-21%	+22%
Combined “Head Twist” (Rotate left + Rotate right + Turn head 360 +Arch down)				
No change	9((50%)	6 (20%)	8 (28%)	6 (33%)
Increase	7(39%)	24 (80%)	9 (31%)	10 (56%)
Decrease	2 (11%)	0 (0%)	12 (41%)	2 (11%)
% change	+28%	+80%	-10%	+44%

Arch down

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Arch down”

In the *NaturO* group, 19/30 animals “increased” and 1/30 “decreased” resulting in a net change of +60%.

In the Both group, 10/18 animals “increased” and 2/18 “decreased” resulting in a net change of +44%.

Arch up

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Arch up”.

In the *NaturO* group, 16/30 animals “increased” and 5/30 “decreased” resulting in a net change of +37%.

In the Both group, 8/18 animals “increased” and 2/18 “decreased” resulting in a net change of +33%.

Press head

All treatment groups had some animals that showed an “increase” and some that showed a “decrease” in the frequency of “Arch down”

In the *NaturO* group, 11/30 animals “increased” and 1/30 “decreased” resulting in a net change of +40%

Summary by treatment

Control - No notable changes overall.

NaturO - 30% or greater net “increase” in all behaviours.

Lignocaine - No notable changes overall.

Both - 30% or greater increase in “Rotate right”, “Arch down” and “Arch up”.

Time series

Figure 3.4.45 shows the total number of times that each specific head-related behaviour was observed in all deer combined during each ten minute time interval during the “pre” and “post” treatment periods. Figure 3.4.46 shows individual data points for deer in each treatment group during each ten minute time interval. The lines on the graph indicate the mean for the group. Graphs were inspected visually for trends. Tests for differences in means and medians were not appropriate because the data were not normally distributed and a relatively small number of animals

demonstrated the behaviour a large number of times while most animals did not demonstrate the behaviour at all. While 2-way ANOVA were performed on the data, the results must be interpreted with caution because of the nature of the data.

Rotate left

During the “pre” treatment period, the frequency of “Rotate left” did not change markedly over time and there were no obvious differences between the treatment groups. During the “post” treatment period, the frequency of “Rotate left” was highest during the first 10 minutes and decreased thereafter, stabilising after 30 minutes, but remaining greater than the pre-treatment count. Most of the increase was due to animals in the *NaturO* and Both groups. Two-way ANOVA of the post treatment data found a significant effect of time, treatment and the interaction of time and treatment. Differences between treatment groups were significant for *NaturO* vs Control during the first 10 minutes and for *NaturO* vs Lignocaine during the first 20 minutes.

Rotate right

During the “pre” treatment period, the frequency of “Rotate right” did not change markedly over time and there were no obvious differences between the treatment groups. During the “post” treatment period, the frequency of “Rotate right” was highest during the first 10 minutes and decreased thereafter. Most of the increase was due to animals in the *NaturO* group. Two-way ANOVA of the post treatment data found a significant effect of time, treatment and the interaction. Differences between treatment groups were significant for *NaturO* vs Control during the first 10 minutes and for *NaturO* vs Lignocaine during the first 20 minutes.

Turn head 360

During the “pre” treatment period, the frequency of “Turn head 360°” remained fairly constant and was low. During the “post” treatment period, the frequency of “Turn head 360°” was highest during the first 10 minutes and dropped markedly thereafter, returning to levels similar to the “pre” treatment period by 30 to 40 minutes after the start of recording. Most of the increase was due to increases in the *NaturO* group. 2-way ANOVA of the “post” treatment values found that there was a significant effect of treatment and the interaction of treatment with time but not time on its own. Differences between treatment groups were significant for *NaturO* vs Control during the first 10 minutes only, for *NaturO* vs Lignocaine during the first 20 minutes and for *NaturO* vs Both during the first 10 minutes only.

Arch head down

During the “pre” treatment period, the frequency of “Arch head down” was variable and appeared to increase slightly during the 60 minutes, although this trend was not significant. During the “post” treatment period, the frequency of “Arch head down” was highest during the first 10 minutes and returned to levels similar the the “pre” treatment period by 10-20 minutes after the start of recording. Most of the increases were due to increases in the *NaturO* group during the first 10 minutes. There was one deer in the Lignocaine group and one or two in the Control group that

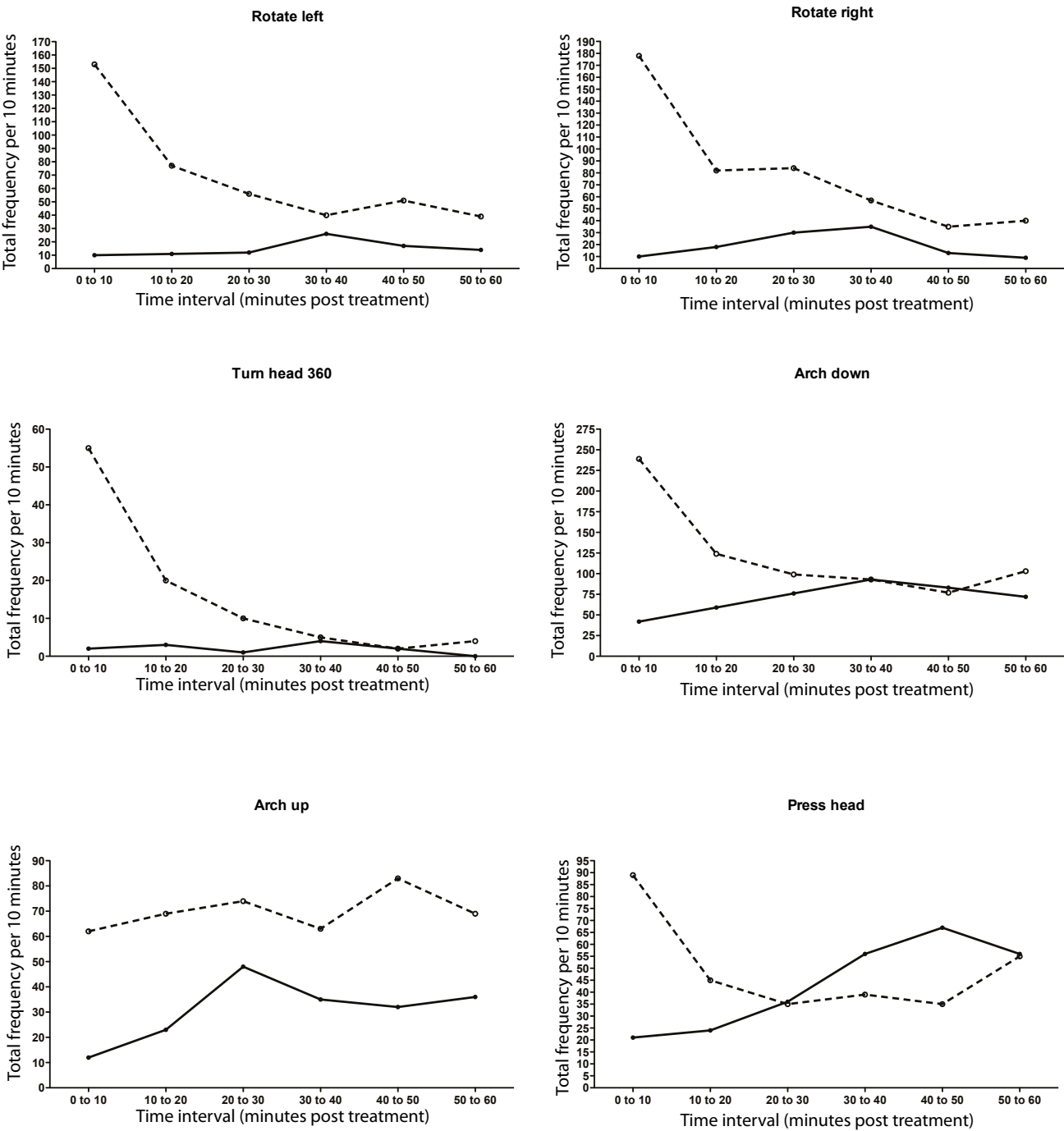


Figure 3.4.45 Time series plots of the total number of times each specific head related behaviour was seen across all animals during each ten minute interval from the start of video observations in pens. “Pre” treatment observations are shown with a solid line and “post” treatment with a dashed line.

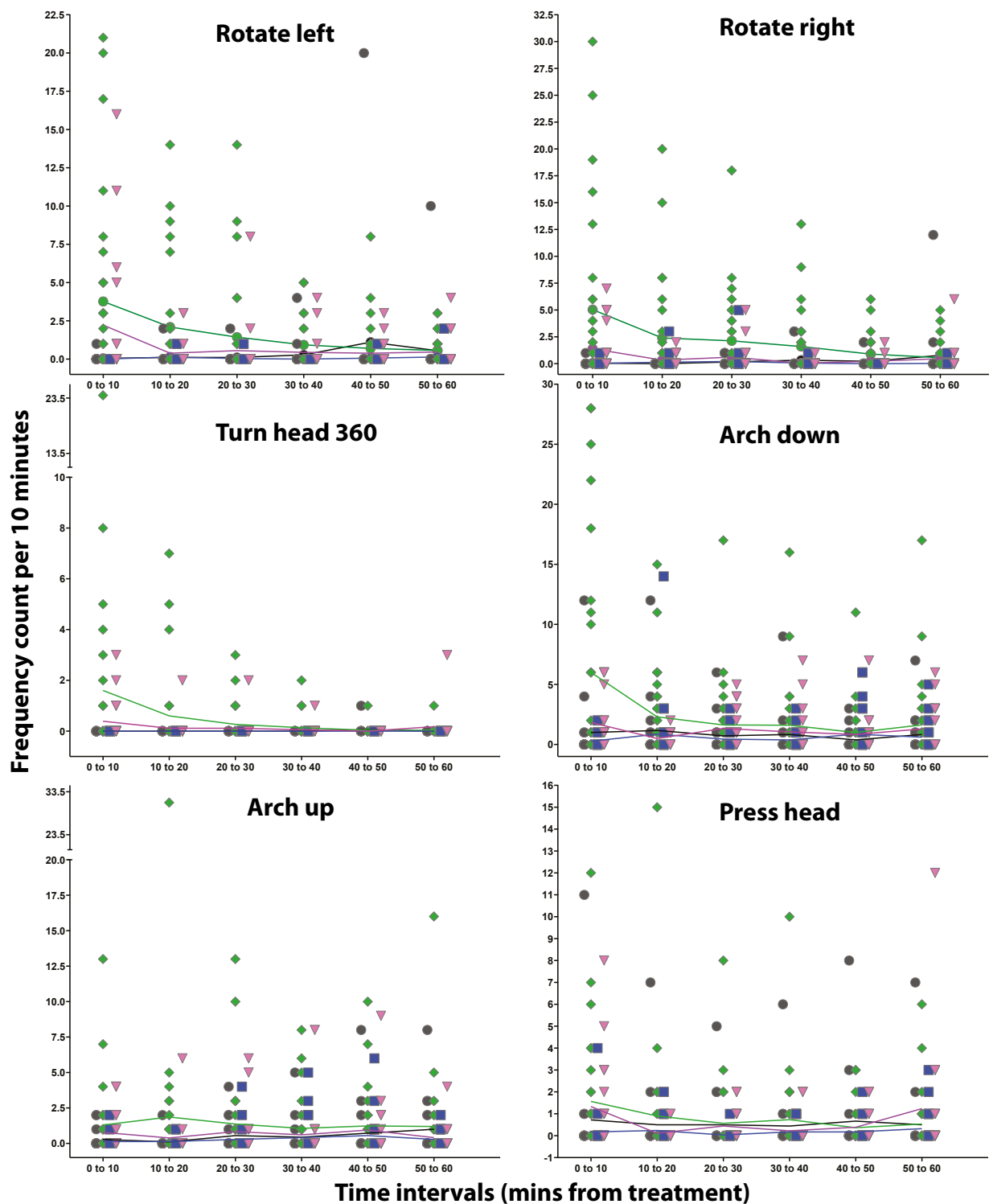


Figure 3.4.46 Time series plots of the number of times that each deer in each treatment group demonstrated “Specific head related behaviours” during each ten minute interval of one hour of observation “post” treatment with either control (black), NaturO™ rings (green), lignocaine (blue) or NaturO on one antler and lignocaine on the other (“both”) (pink). Lines of corresponding colour indicate the mean for each group although the data are not normally distributed.

also had a relatively high frequency of “Arch head down” during the post treatment period. Two-way ANOVA found that treatment, time and the interaction of treatment and time were all had a significant effect on the variation in the data. Between treatment differences were significant for *NaturO* vs Control during the first 10 minutes only, for *NaturO* vs Lignocaine during the first 10 minutes only and for *NaturO* vs both during the first 10 minutes only.

Arch head up

During the “pre” treatment period, the frequency of “Arch head down” was variable and tended to rise during the first 30 minutes and then stabilise. This trend was not significant. During the “post” treatment period, the pattern over time was similar although it was higher for all time periods. The increase was mainly due to increases in a few deer in the *NaturO* group although deer from the Both, Lignocaine and Control groups occasionally had an increased frequency of “Arch head up” as well. Two-way ANOVA found that the effect of treatment was significant but time and the interaction of treatment and time were not. Post ANOVA tests did not show any significant differences between treatments at any of the 10 minutes time intervals.

Press Head

During the “pre” treatment period, the frequency of “Press head” was variable and tended to start low and rise during the first 30 minutes. This trend was not significant. During the “post” treatment period, the reverse was observed with the frequency being highest in the first 10 minutes and decreasing to similar levels to “pre” treatment recordings by 10-20 minutes after the start of recording. Higher levels during the first 10 minutes were mainly due to increases in a few animals in the *NaturO* group. After 10 minutes, one or two deer in the *NaturO* group and one or two deer in the Control group had increased frequency of “Press head” and during the last 10 minutes, one deer in the Both group also had increased frequency of “Press head”. 2-way ANOVA found that treatment had a significant effect on the variation in the data but time and the interaction of treatment and time did not. Post-ANOVA tests found significant differences between the *NaturO* and Lignocaine groups during the first 10 minutes only.

Head Twisting

Specific head related movements tended to be increased in the *NaturO* group when the pre-treatment period was compared to the post-treatment period. In all of the specific head related behaviours the net proportion of deer in the group that “increased” the behaviour during the “post” treatment period compared to the “pre” treatment period was between +30% and +60%.

“Rotate left”, “Rotate right”, “Turn head 360” and “Arch down” were significantly increased in the “post” treatment period compared to the “pre” treatment period in the *NaturO* group. “Rotate left”, “Rotate right” and “Turn head 360” were significantly greater in the *NaturO* group than in the “Control” and “Lignocaine” groups during the “post” treatment period.

The four behaviours “Rotate left”, “Rotate right”, “Turn head 360” and “Arch down” were combined to give an overall “Head twist” score.

Data distribution for Head twist

Overall the total number of deer that demonstrated “Head Twist” was 57 (60%) and 79 (83%) during the “pre” treatment and “post” treatment periods respectively. This difference was statistically significant ($p=0.0011$). The mean number of time that deer demonstrated “Head twist” was 6.4 and 17.0 for pre and post treatment periods respectively. The range was 0- 78 and 0-196 and the median was 4 and 8 for the “pre” and “post” treatment periods respectively. The data were not normally distributed so Wilcoxin signed ranked test was performed. The medians for the “pre” and “post” treatment periods were significantly different ($p=0.002$). A frequency histogram of “Head twist” is shown in figure 3.4.47. In this graph all deer from all treatment groups are combined with “pre” and “post” treatment data shown separately.

Correlation pre vs post

Figure 3.4.48 shows an individual deer scatterplot of “pre” (x-axis) vs “post” (y-axis) frequencies of “Head twist”. Different treatment groups are marked with a different coloured symbol. Both axis are to the same scale. There was not a significant correlation between the “pre” treatment recordings and the “post” treatment recordings indicating that deer did not have a tendency to display “Head twist” behaviour at a similar frequency on different occasions.

Comparisons of “Head twist” within and between treatment

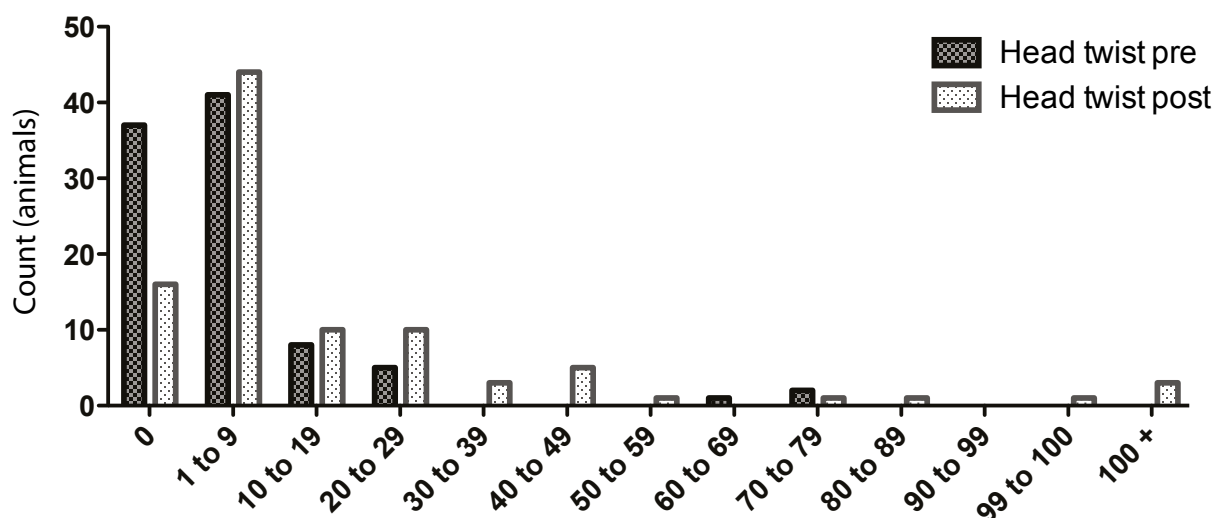


Figure 3.4.47 Histogram of the number of times that deer demonstrated the specific head related behaviours that were combined to make the “Head Twist” behaviour during one hour of video recording before (“pre”) and after (“post”) treatment with either no treatment, lignocaine ring blocks, NaturO™ rings or a combination of these. All treatment groups are combined. “Pre” and “post” values are shown separately.



groups

Figure 3.4.49 shows individual data plots of the frequency of “Head Twist” for deer in each treatment group during the “pre” and “post” treatment recording periods. Non parametric one-way ANOVA found that the medians varied significantly ($p < 0.0001$).

Pre vs Post within treatment groups

In the *NaturO* group, “Head twist” was demonstrated more often during the “post” treatment period than the “pre” treatment period. $p < 0.0001$

In the Both group, “Head twist” was demonstrated more often during the “post” treatment period than the “pre” treatment period. $p = 0.016$.

In the Lignocaine group, “Head twist” was demonstrated more often during the “pre” treatment period than the “post” treatment period. $p = 0.043$

Comparisons between groups

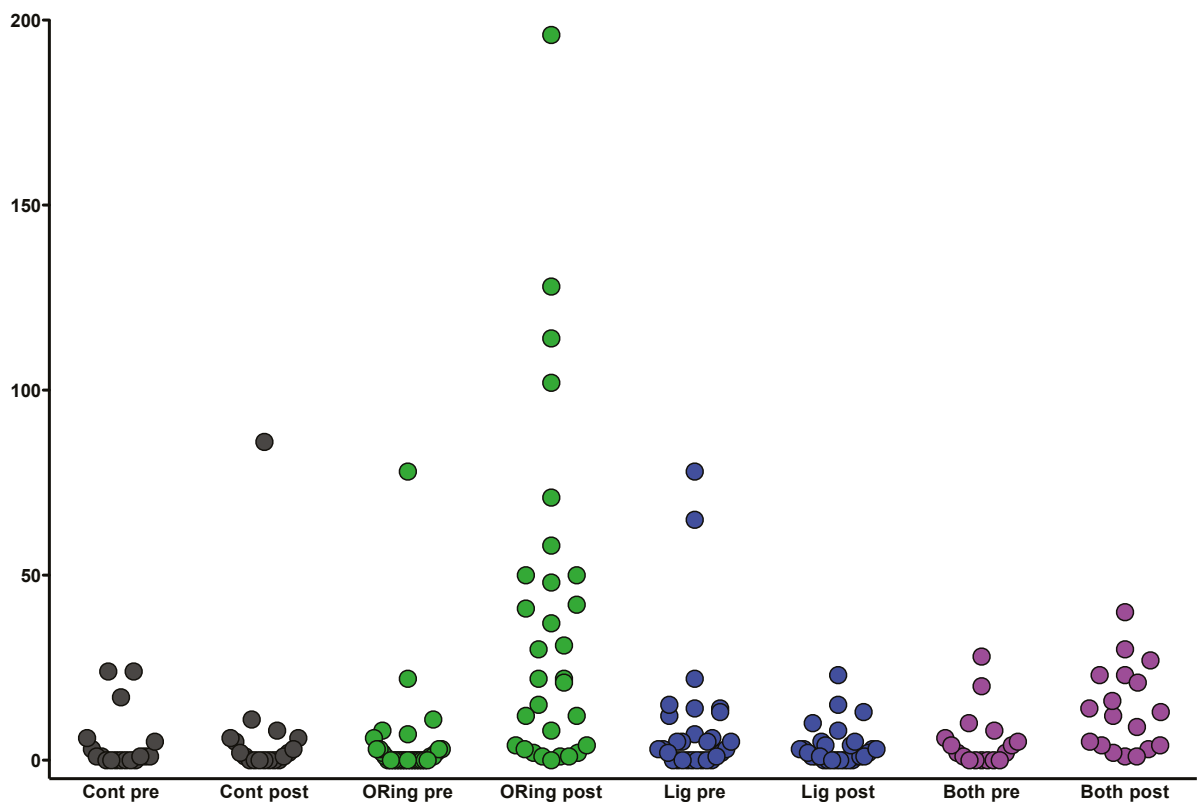


Figure 3.4.49 Individual value data plots of the number of times that one-year-old male deer demonstrated different head position behaviours combined as “Head twist” during one hour of video recording before “pre” or after “post” treatment with a combination of either NaturO™ rings, Lignocaine ring blocks, no treatment (control) or a lignocaine ring block on one antler and NaturO™ ring on the other (both).

Pre treatment

During the “pre” treatment period, Deer in the Lignocaine group demonstrated “Head twist” more often than deer in the *NaturO* group. $p=0.049$

Post treatment

Deer in *NaturO* demonstrated “Head twist” more often than the Control group ($p=0.0002$)

Deer in *NaturO* demonstrated “Head twist” more often than the Lignocaine group ($p<0.0001$)

Deer in Both demonstrated “Head twist” more often than the Control group ($p=0.0019$)

Deer in Both demonstrated “Head twist” more often than the Lignocaine group ($p=0.0003$)

Difference (Post-Pre)

Figure 3.4.50 shows the “difference” (“post”-“pre”) in the frequency of the combined head twist

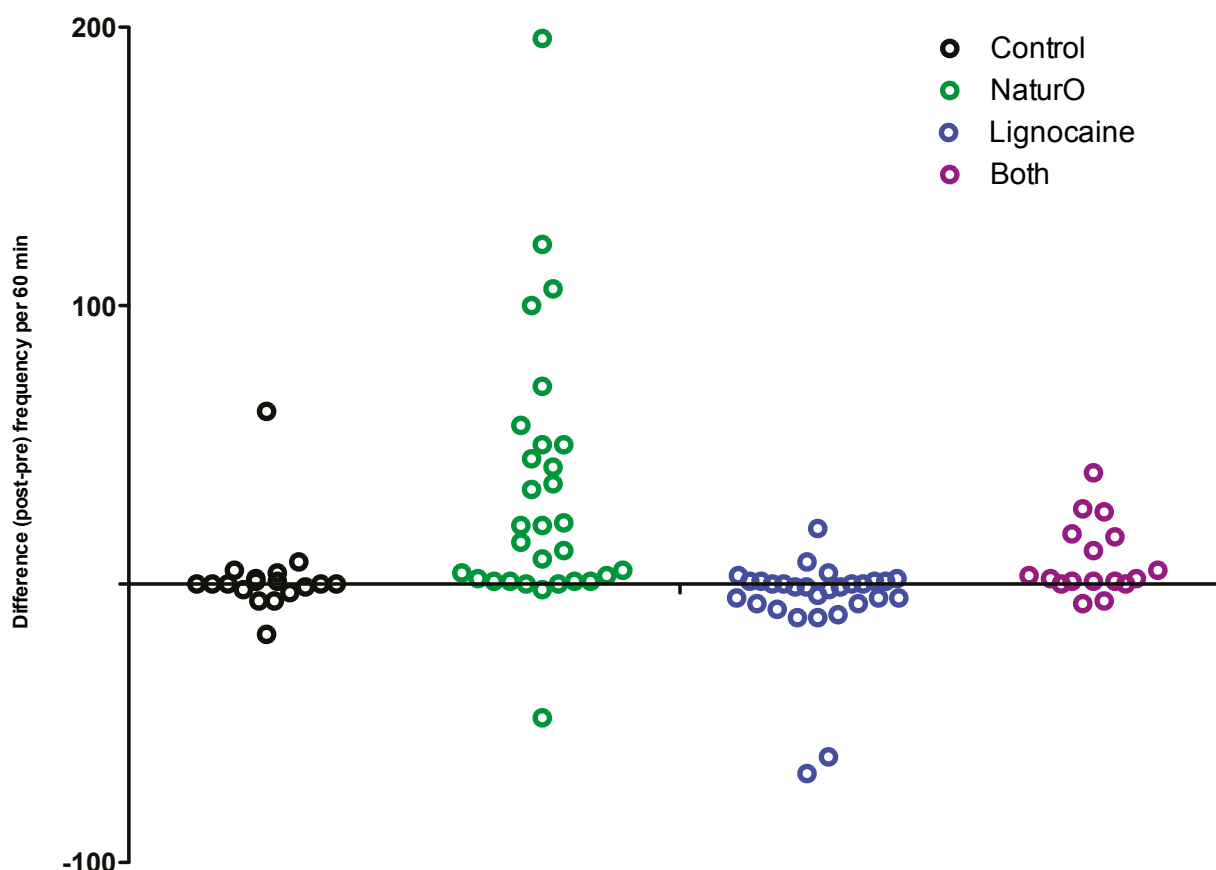


Figure 3.4.50 Individual data plot of the “difference” (“post” - “pre”) in the number of times that one-year-old male deer demonstrate specific head related event behaviours combined to give a “Head twist” behaviour score during one hour of video recording after treatment with either No treatment (Control), *NaturO*TM rings, lignocaine ring blocks or a *NaturO*TM ring on one antler and lignocaine ring block on the other (both).

behaviour for all deer in each treatment group. Mann-Whitney non-parametric t-tests were performed to test for a difference between each treatment group.

Deer in the *NaturO* group had a greater positive “difference” than deer in the Control group. $p=0.0019$

Deer in the *NaturO* group had a greater positive “difference” than deer in the Lignocaine group. $p<0.0001$

Deer in the *NaturO* group had a greater positive “difference” than deer in the Both group. $p=0.0427$

Deer in the Both group had a greater positive “difference” than deer in the Lignocaine group. $p=0.0047$

Proportion of deer than increased or decreased Head twist.

The proportion of animals that had a double (“increase”) or halving (“decrease”) in the frequency of “Head twist” is shown in Table 3.4.15 (previously shown) for deer in each treatment group.

All treatment groups had some animals that showed an “increase” and all treatment groups except *NaturO* had some that showed a “decrease” in the frequency of “Head twist”

In the *NaturO* group, 24/30 animals “increased” and 0/30 “decreased” resulting in a net change of +80%

In the Both group, 10/18 animals “increased” and 2/18 “decreased” resulting in a net change of +44%

Time series

Figure 3.4.51 shows the total number of times “Head twist” was observed during each 10 minute time interval across all deer during the “pre” treatment period and during the “post” treatment period in the top graph. In the middle and lower graphs, individual counts of “head twist” from each deer are shown with treatment group for each animal indicated by a different symbol. The middle graph shows values during the “pre” treatment period and the lower graph shows values during the “post” treatment period. During the “pre” treatment period, the frequency of “head twist” was fairly constant. Higher values were mainly contributed by a few deer in the lignocaine group and one or two in other treatment groups at different times. During the “post” treatment period, the total frequency of “Head twist” was highest during the first 10 minutes and decreased sharply by 20 minutes. After 20-30 minutes the frequency of “Head twist” during the “post” treatment period was slightly higher than during the “pre” treatment period although this difference

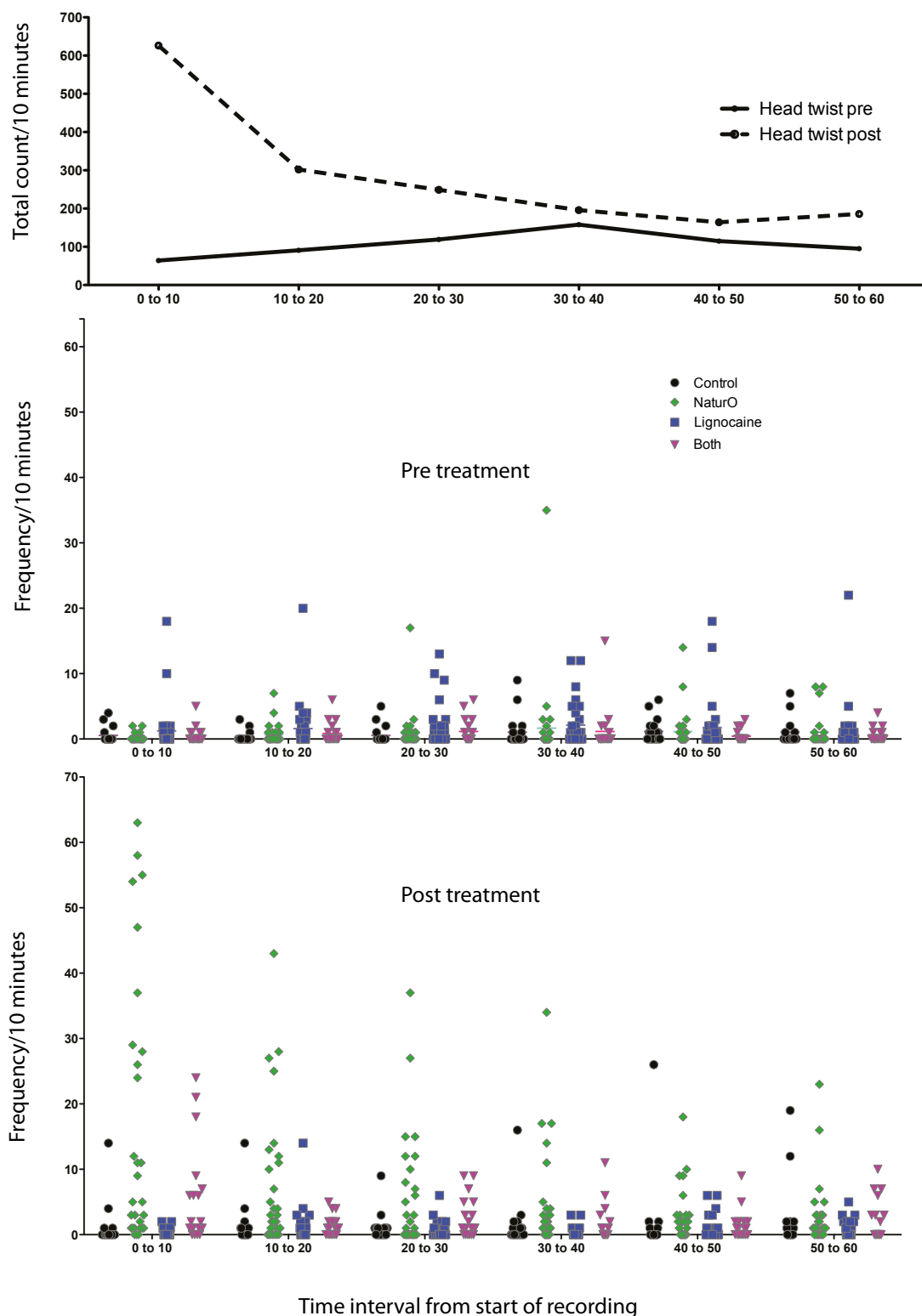


Figure 3.4.51 Time series plots of the frequency of combined specific head related behaviours as “Head twist”. Top graph: Total number of times that “Head twist” was observed in one year old male deer in all treatment groups during each 10 minute interval after the start of video recording for one hour without (pre) and after treatment (post). Middle and lower graphs: Number of times that each deer in each treatment group demonstrated “Head twist” during each ten minute interval during the “pre” treatment period (middle graph) and “post” treatment period (lower graph). Treatment was either control (black), NaturO™ rings (green), lignocaine (blue) or NaturO on one antler and lignocaine on the other (“both”) (pink).

could not be tested statistically. During the first 10 minutes of the “post” treatment period, the increase in frequency of “Head twist” was mostly due to an increase in a large proportion of deer in the *NaturO* group as well as several in the Both group. After 10 minutes the number of deer with increased frequency of “Head twist” in the Both group declined sharply, whereas some deer in the *NaturO* group remained above “pre” treatment levels for 30 to 40 minutes.

Statistics Summary

The table (Table 3.4.13) on the following pages are a summary of the statistical analyses for all behaviours studied in this chapter.

Discussion

For a full discussion of the methods and results in this experiment please refer to the discussion section at the end of this chapter.

Conclusion

*NaturO*TM rings altered the behaviour of yearling male red deer during video observation for specific head related event behaviours. *NaturO*TM rings did not affect the frequency or proportion of time spent in common behaviours observed in deer including head and body position and activity. The duration of the effect of the *NaturO*TM rings on behaviour modification was between 10 and 20 minutes. After 10 minutes the frequency of specific head related behaviours declined.

Table 3.4.13 Statistical Summary of the data distribution and statistical tests applied to data collected relating to behaviours observed of one-year-old male deer during video observation for one hour without treatment (pre) or with treatment (post) with no treatment (control), NaturO™ rings, Lignocaine ring blocks or a NaturO™ ring on one antler and lignocaine ring block on the other (both)

1. The Behaviour in question
2. Did the data pass a D'Agostino and Pearson Omnibus Normality test?
3. The proportion of animals that demonstrated the behaviour at least once during the “pre” treatment period
4. The proportion of animals that demonstrated the behaviour at least once during the “post” treatment period
5. The difference between the proportion positive pre and post. And the result of a Z-test for equal proportions
6. Mean (normally distributed data) and median (non-parametric data) and Range of results (per 60 minutes) for all animals combined during the “pre” recording
7. Mean (normally distributed data) and median (non-parametric data) and Range of results (per 60 minutes) for all animals combined during the “post” recording
8. Test for a significant difference in mean (paired t-test) or median (Wilcoxin matched-pairs sign ranked

State Whole Body Position Behaviours (proportion of time)							
1	2	3	4	5	6	7	8
Behaviour	Is data normally distributed?	“Pre” %+ve	“Post” % +ve	Difference proportion positive post-pre (all)	“Pre” Prop/Freq Mean (median) Range	“Post” Prop/Freq Mean (median) Range	Pre vs Post overall comparison of means or medians
Standing	yes	100	100	No change NS	052 (0.52) 0.07-0.83	0.47 (0.49) 0.03-0.92	NS
Walking	no	97	98	+1% NS	0.06 (0.03) 0.00-0.34	0.09 (0.06) 0.00-0.45	pre < post p=0.04
Pacing	yes	100	100	No change NS	0.37 (0.37) 0.08-0.72	0.39 (0.39) 0.002-0.77	NS
Walking Backwards	no	66	68	+2% NS	0.019 (0.003) 0.00-0.24	0.023 (0.0035) 0.00-0.23	NS
Circling	no	5	17	+12% (p=0.01)	0.0003 (0.000) 0.000-0.012	0.0027 (0.000) 0.00-0.06	pre < post (p=0.003)
Lying down	no	8	4	-4% NS	ND	ND	ND
Running	no	9	9	no change NS	ND	ND	ND
Jumping	no	10	14	+4% NS	ND	ND	ND

test) between “pre” and “post” treatment recordings for all animals

9. ANOVA to test for difference between treatment groups

10. Test for significant difference in the mean (paired t-test) or median (Wilcoxin matched pairs sign ranked test) between “pre” and “post” treatment recordings within each treatment group. Significant differences only are shown in the table.

11. Test for significant differences between treatment groups during the “pre” treatment period. Post ANOVA comparisons

12. Test for significant differences between treatment groups during the “post” treatment period. Post ANOVA comparisons

13. Proportion of animals in each treatment group that either increased (doubled) or decreased (halved) the frequency of the behaviour during the post treatment period compared to the pre treatment period. Result only shown if equal to or greater than 30% net change.

14. Identified trends over time during each 10 minute time interval of the recording period

15. Pearson R or Spearman R test for significant correlation between “pre” and “post” treatment values for individual animals

NS=Not significant. ND = not done

9	10	11	12	13	14	15
ANOVA - Do means vary signifi- cantly?	Pre vs post within treats	between treat- ments (pre)	Between treatments (post)	Proportion change	Time trends	Significant correlation pre vs post?
No	Both: pre > post (p=0.013)	NS	NS	Nil	tend to decrease after 30 mins	yes p=0.0034
No	Both: pre < post (p=0.002)	NS	NS	Both +67%	slight increase with time	yes p<0.0001
Yes p=0.0004	NS	NaturO< Cont (p=0.04) NaturO< Lig (p=0.007) NaturO<Both (p=0.002)	NaturO<Lig (p=0.003) NaturO<Both (p=0.0009)	Nil	nil	yes p=0.046
No	NS	NS	NS	Nil	nil	yes p<0.0001
No	NS	NS	NS	Nil	nil	no
ND	ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND	ND
ND	ND	ND	ND	ND	ND	ND

State Head Position Behaviours (proportion of time)							
1	2	3	4	5	6	7	8
Behaviour	Is data normally distributed?	“Pre” % +ve	“Post” % +ve	Difference proportion positive post-pre (all)	“Pre” Prop/Freq Mean (median) Range	“Post” Prop/Freq Mean (median) Range	Pre vs Post overall comparison of means or medians
Head straight	yes	100	100	no change NS	0.44(0.45) 0.18-0.70	0.45(0.46) 0.17-0.80	NS
Head left	yes	100	100	no change NS	0.25 (0.24) 0.10- .44	0.22 (0.21) 0.07-0.44	NS
Head right	yes	100	100	no change NS	0.21 (0.21) 0.08-0.38	0.21 (0.21) 0.05-0.35	NS
Head high	no	89	93	+4% NS	0.015(0.007) 0-0.10	0.012(0.007) 0-0.079	NS
Head low	no	100	100	no change NS	0.085(0.07) 0.003-0.040	0.084(0.07) 0.002-0.39	NS
Event Head Position Behaviours (frequency per 60 minutes)							
1	2	3	4	5	6	7	8
Behaviour	Is data normally distributed?	“Pre” % +ve	“Post” % +ve	Difference proportion positive post-pre (all)	“Pre” Prop/Freq Mean (median) Range	“Post” Prop/Freq Mean (median) Range	Pre vs Post overall comparison of means or medians
Head straight N	yes	100	100	No change NS	237 (236) 83-394	250 (260) 123-453	NS
Head left N	yes	100	100	No change NS	136 (131) 53-234	133 (136) 54-218	NS
Head right N	yes	100	100	No change NS	132 (131) 61-246	140 (134) 33-263	NS
Head high N	no	89	93	+4% NS	10.8 (8.0) 0-41	10.1 (7.0) 0-48	NS
Head low N	no	100	100	no change NS	71 (63) 6-245	72 (56) 7-280	NS

9	10	11	12	13	14	15
ANOVA - Do means vary signifi- cantly?	Pre vs post within treats	between treat- ments (pre)	Between treatments (post)	Prop change (Only shown if >= 30% net change)	Time series	Significant correlation pre vs post?
No	NS	NS	NS		Increase with time	yes p=0.017
No	NS	NS	NS		slight decrease over time	yes p<0.0001
No	NS	NS	NS		slight decrease over time	yes p<0.0001
No	NS	Cont<Lig p=0.01	NS	Lig: - 31%	nil	yes p=0.0015
No	NS	NS	NS	Lig: -31%	nil	yes p=0.0002
9	10	11	12	13	14	15
ANOVA - Do means vary signifi- cantly?	Pre vs post within treats	between treat- ments (pre)	Between treatments (post)	Prop change (Only shown if >= 30% net change)	Time series	Significant correlation pre vs post?
NS	NS	NS	NaturO<Both (p=0.019)			yes p=0.0292
NS	NS	NS	NS			yes p=0.0007
NS	Both: pre<post p=0.038	Lig > NaturO (p=0.0066)	NaturO<Lig (p=0.017) NaturO<Both (p=0.0028)			yes p<0.0001
NS	NS	NS	NS	Lig -38%		yes p=0.0003
NS	NS	Cont>NaturO (p=0.048) Lig > NaturO (p=0.001)	NS			yes p=0.0012

Event: Non-specific (frequency per 60 minutes)							
1	2	3	4	5	6	7	8
Behaviour	Is data normally distributed?	"Pre" % +ve	"Post" % +ve	Difference proportion positive post-pre (all)	"Pre" Prop/Freq Mean (median) Range	"Post" Prop/Freq Mean (median) Range	Pre vs Post overall comparison of means or medians
Look up	No	39	33	-6% NS	2.69 (0) 0-31	0.78 (0) 0-7	Pre>post (p=0.014)
Nose to ground	No	98	96	-2% NS	31.2 (21) 0-118	29.7 (25) 0-125	NS
Lick the walls	No	93	95	+2% NS	15.3 (12) 0-62	13.9 (11) 0-75	NS
Flick tongue	No	100	100	No change NS	27 (24) 1-68	35 (31) 3-171	Pre<Post (p=0.0006)
Flick ears	No	85	78	-7% NS	4.36(2.0) 0-22	4.0 (2) 0-31	NS
Jump	No	10	14	+4% NS	0.49(0) 0-21	0.40(0) 0-9	NS
Groom self	No	31	27	-4% NS	1 (0) 0-18	0.52(0) 0-6	NS
Groom other	No	52	29	-23% (p<0.0012)	2.2 (1) 0-24	0.54 (0) 0-7	p<0.0001
Scratch	No	14	11	-3% NS	0.46(0) 0-18	0.25 (0) 0-10	NS
Shake body	No	73	57	-16% (p=0.021)	1.04(1) 0-4	0.91(1) 0-6	NS
Stand up	No	6	8	+2% NS	ND		
Bite	No	2	3	+1% NS	ND		
Bitten	No	2	3	+1% NS	ND		

9	10	11	12	13	14	15
ANOVA - Do means vary signifi- cantly?	Pre vs post within treats	between treat- ments (pre)	Between treatments (post)	Prop change (Only shown if >= 30% net change)	Time series	Significant correlation pre vs post?
No	Cont: Pre>Post (p=0.041)	NS	NS	Cont: -39%		no
No	NS	NS	NS	Lig: -31%		yes (p<0.0001)
No	NS	NS	NS	Both: -39%		no
No	Cont: Pre<Post (p=0.0056) NaturO: Pre<Post (p=0.035)	NS	NS	NaturO +30%		yes (p<0.0001)
No	Lig Pre > Post p=0.05	NS	NS	Lig: -42%		yes (p<0.0001)
No	NS	NS	NS			
yes p=0.046	Lig: Pre>Post p=0.0078	NS	NS			no
yes p=0.024	Lig Pre>Post p=0.0038 Both Pre > Post p=0.043	NS	NS	Lig -34% Both -33%		no
No	NS	NS	NS			no
No	NaturO: Pre > Post p=0.0008	NS	NaturO>Cont p=0.025 NaturO<Lig p=0.042 NaturO<Both p=0.034	NaturO -43%		no
ND						
ND						
ND						

Event: Specific head related (Frequency per 60 minutes)							
1	2	3	4	5	6	7	8
Behaviour	Is data normally distributed?	“Pre” %+ve	“Post” % +ve	Difference proportion positive post-pre (all)	“Pre” Prop/Freq Mean (med) Range	“Post” Prop/Freq Mean (med) Range	Pre vs Post overall comparison of means or medians
Rotate left	no	23	38	+15% (p=002)	0.80 (0) 0.0-16	3.87 (0) 0.0-52	p=0.0002
Rotate right	No	25	41	+16% (p=0.018)	1.13 (0) 0.0-17	4.65 (0) 0.0-86	p=0.0014
Turn head 360	No	4	16	+12% (p=0007)	0.13 (0) 0.0-9	0.99 (0) 0.0-33	p=0.0076
Arch down	No	46	69	+23% (p=0.009)	4.36 (0) 0.0-62	7.40 (2) 0-88	p=0.04
Arch up	No	39	54	+15% (p=0.039)	1.58 (0) 0.0-18	3.82 (1) 0-73	p=0.028
Press Head	No	41	46	+5% NS	2.7 (0) 0.0-46	3.1 (0) 0-45	NS
Head Twist	No	60	83	+ 23% (P=0.0011)	6.4 (1.5) 0.0-78	17.0 (4.0) 0.0-196	p=0.02

9	10	11	12	13	14	15
ANOVA Do means vary signifi- cantly?	Pre vs post within treats	between treat- ments (pre) Mann- Whit- ney	Between treatments (post) Mann-Whit- ney	Prop change (Only shown if >= 30% net change)	Time series	Significant correlation pre vs post?
yes p<0.0001	NaturO pre<post p=0.0001 Both pre < post p= 0.032	NS	NaturO > Cont p=0.0002 NaturO >Lig p<0.0001 Both > Con p=0.034 Both > Lig p=0.016	OR +60%		No
yes p<0.0001	NaturO pre <post p=0.0004 Both pre > post p=0.046	NS	NaturO > Cont p=0.0005 NaturO >Lig p<0.0001 Both > Cont p=0.014 Both > Lig p=0.0011	OR +53% Both +39%		No
yes p<0.0001	NaturO pre < post p=0.0001 Both pre <post p=0.034	NS	NaturO>Con p=0.0002 NaturO>Lig p<0.0001 Both >Con p=0.034 Both > Lig p=0.016	OR +30%		No
yes p=0.0008	NaturO pre < post p=0.0007 Both pre < post p=0.022	Lig > NaturO p=0.0063	NaturO>Cont p=0.045 NaturO> Lig p=0.019 Both > Lig p=0.027	OR +60% Both +44%		No
yes p=0.0037	NaturO pre <post p=0.025 Both pre < post p=0.012	NS	NaturO>Con p=0.016 NaturO>Lig p=0.0047	OR +37% Both +33%		No
yes p=0.012	NS	NS	NaturO>Cont p=0.041 NaturO> Lig p=0.006 Both > Cont p=0.037 Both > Lig p=0.0064	OR +40%		No
yes p<0.0001	NaturO pre < post p<0.0001 Lig pre > post p=0.043 Both pre < post p=0.016	Lig > NaturO p=0.049	NaturO > Cont p=0.0002 NaturO >Lig p<0.0001 Both > Cont p=0.0019 Both > Lig p=0.0003	OR +80% Both +44%		No

The degree of noxiousness of the *NaturO*TM rings can not be determined in this study and the presence of a velcro band applied loosely around the antler pedicle also appeared to alter these specific head related behaviours significantly but to a lesser extent than the *NaturO*TM rings. Lignocaine ring block did not alter behaviour.

Discussion of Chapter 3

In the experiments in this chapter application of a *NaturO*TM ring one hour prior to velvet antler removal reduced the EEG response to velvet antler removal but did not completely prevent it in all deer and we were unable to detect a difference in the behaviour of yearling stags, during antler removal, that had previously been treated with either a *NaturO*TM ring or lignocaine ring block. *NaturO*TM ring application causes an EEG response in some deer that was different from deer administered with lignocaine and the frequency of some behaviours was increased in some deer when *NaturO*TM rings were applied. There is one other published experiment (Nicol et al, 2009) on the efficacy of the *NaturO*TM ring for providing analgesia at the time of antler removal and to our knowledge these experiments are the first that have used EEG to assess velvet antler pain in yearling deer and also the first that have specifically investigated the potential noxiousness of the *NaturO*TM rings during application. While our behavioural observations concur with those of Nicol et al (2009), the overall result suggest the *NaturO*TM rings are less effective than a correctly applied lignocaine ring block and may be uncomfortable during application.

The two methods used in the four experiments were EEG and behaviour. These proved to be appropriate under different circumstances. In the first two experiments which aimed to determine whether the deer were still sensitive to pain due to antler removal after one hour of *NaturO*TM ring application, EEG was a sensitive method and was able to detect differences between treatment groups. Acute withdrawal behaviour response was less specific than EEG and this is probably because one year old deer are likely to react to the handling experience and become stressed, masking the movements that may have occurred in response to antler cutting. For the experiments in Chapters 3.3 and 3.4, which aimed to determine whether the *NaturO*TM ring was noxious during application, behaviour proved to be more appropriate than EEG. EEG is most useful for detecting acute changes, whereas the application of the *NaturO*TM ring had ongoing effects on behaviour that lasted several minutes.

Behavioural studies

There were several differences in the design of the behaviour studies in chapters 3.2 and 3.4 which are likely to have resulted in the difference in the success of these experiments. These warrant discussion so that future experiments of this type can be designed appropriately. These topics have rarely been discussed with respect to large animal behaviour studies.

Selection of animals

The most important criteria for selection of animals is that they are relevant to the experimental question but they must also be suitable for the experimental situation. For testing the *NaturO*TM ring it was necessary to use yearling stags as these are the only animals that the *NaturO*TM ring is used on in practice. If the experiments here were conducted on adult stags, a different set of behaviours may have been observed. While Malony et al (1993) observed similar behaviours in lambs of different ages to the same castration or tail docking method, McCracken et al (2010) observed differences in behaviours of lambs during tail docking that had been castrated at different ages suggesting that both the age of the animal and its previous experiences influence its behavioural response during noxious experiences. Yearling stags proved more difficult to handle in the crush than the adult stags in Chapter 2, with a greater tendency to show confounding background behaviour making it difficult to assess a behaviour outcome in experiment 3.2, where a definite outcome (withdrawal or no withdrawal) could not be determined in 22% of antler removals. A major problem encountered in that experiment was the difference in behaviour between stags from different source farms with the deer born and reared on the Massey University farm more likely to show confounding background behaviour than those bought in from a different farm for the experiment (43% vs 3% not determinable, respectively). It would have been preferable to have all of the deer in that experiment reared together from birth and having the same lifetime experiences with respect to yarding and handling. The deer in experiment 3.4 were from a single cohort that had been specifically handled for the experiment and although there were differences between individuals in that experiment, they were less pronounced than the differences between cohorts in experiment 3.2.

Stags reared on the Massey farm tended to move more once restrained in the crush. They had a greater number of responses to handling and attempted to struggle even when not being handled. Covering the eyes with a towel or use of a halter did not aid differentiation of a withdrawal response. In contrast the animals that were bought in remained very still once restrained in the crush and demonstrated very few movements during cutting of the antler regardless of analgesic used. This may indicate that both treatments effectively eliminated pain, or that these animals did not express pain behaviour due to a fear-freeze response.

These two groups of animals were used for a concurrent study using EEG to compare *NaturO*TM ring and local anaesthetics (Chapter 3.1) and a similar discrepancy in behaviour was observed during catheterisation of the facial vein. The Massey reared stags threw their heads continuously and invariably required manual restraint by at least one person, whereas the bought in animals that were much more fearful in the paddocks and yards did not require any additional physical restraint during catheterisation.

The effect of individual temperament and cultural differences between groups on ability to interpret a pain response was further assessed by investigating the responses in fifty animals that had both antlers removed. Twelve demonstrated an indeterminable response on removal of the first antler. Of these, 8 (67%) also demonstrated an indeterminable on the second antler which was removed at a later date. Thirty-eight animals had a determinable outcome on the first antler, and of these 4 (11%) were not determinable when the second antler was removed. This indicated that

individual animals were likely to show repeated confounding behaviours on different occasions during restraint, even when a different analgesic was used.

A study investigating the effect of different husbandry procedures on various stress parameters in two and four year old deer (Matthews et al, 1990) found that individual animals were consistently easy or difficult to handle when brought into the crush and that two-year-old deer scored a higher “behaviour score” (more difficult) than four-year-old deer. In preference testing yearling stags, Pollard et al (1991) found that the majority of stags would consistently choose an empty pen over one occupied, whereas a small number of individuals consistently chose the opposite. This result suggests that individual animal temperament and cultural differences may exert confounding influences on behavioural study outcomes and should be taken into consideration when designing such experiments.

Number of animals

A major limitation of the experiment in Chapter 3.4 was the small number of animals in each treatment group and large variation in behaviours between animals. The number used (ten) was similar to other studies in deer and cattle. Pollard et al (1992, 1993, 1994) consistently used eight animals per treatment to investigate the behaviour of deer following velvetting with different analgesic treatments. Eight (McMeekan et al, 1999; Doherty et al, 2007), ten (McMeekan et al, 1998; Graf and Senn 1999; Faulkner and Weary, 2000) or 12 (Stafford et al, 2000) animals per treatment has also been used in calf dehorning studies.

ANOVA analysis of our data when the original 10 treatment groups, with 10 animals per treatment, were compared found no statistically significant difference between any of the treatment groups in any of the behaviours analysed. When groups of similar treatment type were combined, there were significant differences in some behaviours. In order to ensure enough animals in the control group for statistical comparison, the no-treatment (Velcro band only) control and the group with both *NaturO*TM rings and lignocaine on both antlers were combined. This is not an ideal way to analyse the data and may decrease the validity of the results for this group. As each animal acted as its own control, the data were still able to be analysed based on pre and post treatment changes for each animal and the comparisons between *NaturO*TM ring and Lignocaine treatment groups could be compared directly. It would be desirable to repeat this study with a much larger number of animals in each treatment group but this type of study is very time consuming and costly in terms of technical time so the benefit of the additional knowledge that could be gained would have to justify the cost of the study.

Conditioning

Many researchers have used a conditioning or habituation period prior to making observations in response to treatment (E.g. Doherty et al, 2007; Theurer et al, 2012). This is useful to reduce changes in activity that may be related to being in a strange or stressful environment. For example Doherty et al (2007) handled calves around the neck to simulate blood sampling so that the handling would have minimal effect on cortisol levels and any rise could be associated with the treatment under investigation. The experiment in Chapter 3.4 included an habituation period of six

weeks. This was considered particularly important in deer due to their pronounced stress response to yarding and handling. A habituation period was not used in the experiment in Chapter 3.2 because it was anticipated that the behaviours demonstrated during the acute stimulus of antler cutting would not be altered by habituation to handling. In retrospect, conditioning the deer to yarding and crushing and non-painful handling about the head may have reduced the struggle response seen in a large number of Massey-reared animals. Conversely, this may not have been the case as the Massey-reared deer had been handled more than the bought-in deer and they also tended to be the ones that moved more during the procedure, making it difficult to interpret the behaviour during antler removal.

Removal of background stimulus

Remote recording systems are a great advantage to the objectivity of data collection and also preventing behaviours in response to the presence of an observer. Video data collection is now widely used (E.g. Pollard et al, 1992, 1993, 1994; Stafford et al, 2000; Doherty et al, 2007) and has the advantages that data is not affected by the presence of a human observer as well as complete and accurate data collection that removes human error associated with live behaviour scoring. Increased objectivity of data collection with RFID tags and readers was used to determine the position of calves after dehorning with or without meloxicam (Theurer et al, 2012). Another way to remove confounding background stressors would be to use a long-acting tranquilliser and this has proved effective in wapiti deer (Woodbury et al, 2001) for removing confounding due to a stress response to handling. In the experiment in Chapter 3.2, it would have been useful to inject the deer with zuclopenthixol prior to the antler removal observations.

The use of controls

It is essential to have a control group in behavioural studies so that there is a baseline for determining the effect of the treatment. We observed large between animal variation in behaviours both in the efficacy and noxiousness studies. Graf and Senn (1999) described large between animal variation in some behaviours, particularly head-shaking which made statistical analysis difficult. Graf and Senn stressed the importance of performing a non-treatment (simulated) observation on animals prior to the treatment observations to obtain behaviour data for animals that were subjected to the environment and procedures (without treatment) and any differences in behaviour between these observations would therefore be attributable to the treatment. In the noxiousness study in Chapter 3.4, a pre-treatment recording was made on all animals and a relative increase or decrease in the frequency of behaviours was determined for each individual. This was a useful way to analyse the data and has not commonly been described by previous authors. In the efficacy study in Chapter 3.2, no baseline behaviour data were collected prior to antler removal. It would have been useful in that study to record behaviour during a sham antler removal session so that the normal behaviour of each deer was recorded and a comparison could be made to the behaviour seen in response to antler removal. It would not have been possible to completely simulate antler removal but the procedure of restraint, injection (of saline perhaps) and handling of the antlers could have been performed. It would also have been useful to treat each of the two antlers of each animal with a different treatment and compare the behavioural response between antlers for each animal. This could have been done in a cross-over design study but was not done on this occasion due to the

requirement to move animals off the farm in a short time-frame. The experiment conducted by Nicol et al (2009) was designed in such a way with the *NaturO*TM ring applied to one antler and a lignocaine ring block applied to the other antler. The noxious stimulus used in that study was electrical stimulation of the antler followed by a nick test. The data were improved by removing animals that did not respond to electrical stimulation of the antler at 40mA prior to applying the treatment. Nicol et al (2009) also included control animals that did not receive analgesia whereas we did not include controls in the efficacy study as it was considered that the welfare of the deer would be compromised.

Behaviour recording software

Very few behaviour studies in large animal pain (particularly deer) research have described the use of behaviour recording and analysis software. This may be, in part, due to the relative high cost and complexity of behaviour analysis software such as “Observer” (Noldus Information Technology) and may also be due to the relatively straight-forward data analysis when a single behavioural response is being associated with a single trigger such as withdrawal to antler cutting. Other types of behavioural analysis in deer have used behaviour analysis software (e.g. Abeyesinghe and Goddard, 1998). Behaviour analysis software was not used in Chapter 3.2, but was used in Chapter 3.4, because the data were more complex in the latter study and it was preferable to record all of the data once only and review it later if required. This is unlikely to have affected the results in 3.2.

JWatcher

We decided to use JWatcher (Blumstien and Daniel, 2007) software after exploring the possibility on the Internet and communicating with the author of the software, Daniel Blumstein and identifying advantages over other software options. JWatcher is free, relatively small file size and easy to download from the Internet. It is compatibility with Mackintosh and Microsoft computers with a version available for Mac OS “Lion”. Using the JWatcher system it was possible to add new behaviours and start recording them at any time during the scoring procedure without the need for a predetermined set of behaviours at the start. This was especially useful when a new behaviour was seen after several deer had already been scored. The flexible editing features meant that animals that had already been scored could be reviewed and this behaviour manually added at the time that it occurred, using the time-stamp in JWatcher. Data were time stamped from the start of recording for each animal which allowed analysis to be broken down into time periods of any specified duration. This corresponded with the score once-analyse multiple times type of setup and allowed data to be analysed during any specified time period.

The major disadvantage was the incompatibility of video capture and reading software within the equipment available. The video linking feature would have been useful but the time required to convert all videos to a compatible format prevented this from being used. This was able to be overcome in this project however as the technician was able to watch the films on a separate monitor and synchronise the times on the film with the times on JWatcher so that the film did not have to be imported into JWatcher for analysis.

Objective data collection

A technician was trained to record data in the experiment in Chapter 3.4 to improve objectivity and consistency. Roughan and Flecknell (2006) found that a lay-person that was trained to observe and record specific behaviours was better able to determine the analgesic treatment given to animals than an experienced person making a subjective assessment of the animal's behaviour. More recently, Roughan et al (2009) found that behavioural recognition software was also able to accurately predict the treatment group of animals and offer further objectivity to the behavioural scoring. As the main researcher was intimately involved with the project and more likely to be biased in scoring behaviours, it was appropriate to employ a technician that was blind to the treatments and unfamiliar with the animals to score the behaviours. One problem with having a trained technician was the lack of lateral thinking and unique observation. For example, on rewatching some tapes at a later date a behaviour that could be described as chin rubbing was observed by the author. This may be similar to "Head rub" observed by Webster and Matthews (2006). A deer would stand with its head on the back or neck of another deer and run its chin. This tended to be seen more in animals that also showed head rotation and head pressing. Unfortunately it was not included in the original data collection and time prohibited re-running the data collection exercise. There may have been other behaviours that also were not detected by the technician. This demonstrates that it is difficult to individually identify and define every single type of behaviour that an animal shows. Some behaviours may be quite subtle and beyond the detection limits of the video equipment. It is possible that a different observer would identify a different set of behaviours that may be more or less associated with the sensation of a *NaturO*TM ring on the antler pedicles. It is also probable that the more subtle behaviours would be more specific as deer will tend to hide signs of pain, distress or inferiority, especially in the presence of a potential predator but also in the presence of other deer where such weakness may be picked on by the stronger members of the group. Deer with *NaturO*TM rings did not tend to be the victims of aggression any more than any other treatment.

Selection of the behaviours to be included in analysis

It would not be practical to include an exhaustive list of all behaviours demonstrated by animals so a subset of behaviours must be selected. In selecting these behaviours, it is important to consider the nature of the noxious stimulus and the expected duration of the sensation. For the efficacy study (Chapter 3.2), an acute withdrawal response was anticipated but for the noxiousness study (Chapter 3.4), a wide range of behaviours previously described following antler removal (Pollard et al, 1992, 1993, 1994) were expected.

Acute withdrawal behaviour during electrical stimulation of the antler (Wilson et al, 1999; Bartels et al, 2001) and antler removal (Wilson et al, 1999) has been used in yearling deer to determine analgesic efficacy. No previous studies have been found that describe the behaviour of the deer in objective terms or attempt to use more specific criteria for a withdrawal response during velvet antler removal. Wilson et al (1999) scored behaviours (0 to 3) based on criteria relating to the size of the movement and the body parts involved. For example a score 1 involved slight head movement, flinch, muscle tension and eye or facial muscle movement and score 3 involved a whole body flight response. The results from the experiment in Chapter 2 of this thesis and the observations

in the experiment here, suggest that the criteria used by Wilson et al (1999) may not be appropriate for velvet antler pain and that the behaviours described as a score 1 may be more specific for failure of analgesia than those described as score 3, which may be non-specific movements during handling. Wilson et al (1999) combined scores 1-3 as a withdrawal response which leads one to question why these different behaviour criteria were used at all. The full range of behaviours likely to be shown by yearling deer was not known prior to commencing these experiments. Initially it was intended that the behaviours recorded in Chapter 3.2 would be the same as those described in Chapter 2 of this thesis. All of the deer in the study in Chapter 2 were two-years-old or older, whereas all of the deer in the current experiment were less than one year of age. After the experiment was started it became evident that the behaviours that had been quite successful at indicating a withdrawal response in older stags may not be appropriate in rising one-year-old stags. The list of behaviours was modified to suit this age-group of animals. Several of the movements are similar and have only been slightly changed in their description for example head rotate and flinch. The nonspecific movements involving the whole body were divided into a single launch forwards and a continuous struggle. New behaviours included ears down, eye flicker, tongue flick, nose up, head flick and head dip. These behaviours may have been present in some of the older stags in the experiment in Chapter 2 but were not recorded at that time. Over both of these experiments there have been a large number of behaviours observed and only a small number chosen for actual data analysis. In some groups, the behaviours occurred with low frequency so that statistical analysis would not be valid and others might be quite discrete and difficult to detect reliably on video. It is quite probable that these small directional movements are more specific indicators of a response to a particular stimulus. This may be pain, but may equally be noise, light or smell that the observer cannot detect. There has been very little research on the normal range of behaviours in deer and what triggers may cause them. This is an area that warrants further research.

With a relatively large body of evidence that velvet antler removal without analgesia is probably painful, it would be unethical to include a control group of animals in which antlers are removed without the use of analgesia. A control group, with no analgesia, would have allowed specific behaviours demonstrated in response to pain to be identified and helped to determine which behaviours are specific to antler pain and which are non-specific reactions to external stimuli. While some research centres may allow electrical stimulation of the sensitive antler, the validity of an electrical stimulus as a proxy for whole antler removal using a saw has not been shown. The particular behaviours demonstrated during electrical stimulation may differ from those shown during saw cut. Using a behaviour model, the only comparisons that can be made are between a proven analgesic (lignocaine) and the alternative to be tested (in this case, *NaturO*TM ring).

There have been no published studies found on the behaviour of deer in response to pressure on the antler pedicle as would be applied by a *NaturO*TM ring or tight tourniquet. Some studies on the behaviour of deer associated with velvet removal (Pollard et al 1992, 1993, 1994; Matthews et al, 1994) have been published, and there are many publications on the behaviour of calves following dehorning with or without various analgesics. Previous research on the long-term behaviour in calves and deer following different treatments has generally focused on changes in frequency or duration of behaviours that would normally be displayed by the animal while abnormal behaviours have only occasionally been reported. Results using changes in normal behaviour have been variable. Of eight behaviours studied by McMeekan et al (1999) in calves dehorned with or

without local anaesthetic and ketoprofen, four showed significant differences between treatments, being lying down, grazing or rumination, tail flicking and ear flicking. Stafford et al (2000) were unable to identify differences in certain behaviours (lying down, eating, ear flick, tail shake, head shake, rumination, grooming and scratching) of calves immediately following scoop dehorning with or without local anaesthetic and those authors suggested that the particular behaviours studied may not have been relevant to the pain experienced by calves following dehorning. Conversely, Sylvester et al (2004) identified significant differences in tail flicking, head shaking, ear flicking and rumination between calves that were dehorned with or without local anaesthetic. Other “normal” behaviours were not affected and an abnormal behaviour “neck extending” was identified but only seen at low frequencies. In two similar studies investigating the effects of ketoprofen on the behaviour of calves following dehorning, one (Milligan et al, 2004) did not find a difference in behaviours in two-week old calves, while the other (Duffield et al 2010) found differences in ear flicks, total head behaviour and feed consumption in four-week old calves. Theurer et al (2012) found differences in position and feeding behaviours in calves following dehorning with or without meloxicam, using an accelerometer to gather data remotely and objectively, while Faulkner and Weary (2000) found that the frequency of head shaking and ear flicking but no other behaviours was reduced in calves administered ketoprofen compared to those dehorned but not given ketoprofen.

Pollard et al (1992; 1993; 1994) identified changes in behaviour such as ear-flicking, head shaking, nosing the ground, grooming, scratching and tongue flicking in response to velveting with local anaesthetic compared to deer that were not velveted. Application of long acting analgesics did not produce significant patterns of change in the behaviours observed in one study (1994) but did in others (1992, 1993). Some of the behaviours that were found to differ between treatment groups by Pollard et al (1992, 1993) were also recorded in the experiment in Chapter 3.4 and no significant difference were found between treatment groups. If we had relied solely on these behaviours we would not have identified any effect of treatment with the *NaturO*TM ring. Unique behaviours that were not normally seen were better predictors of the treatment than changes in the frequency of normal behaviours. The head-rotating behaviours that we observed have not previously been described in deer. The following is a detailed discussion of the different types of behaviours observed in Chapter 3.4.

Whole body state behaviours

Apart from the “Both” treatment group which tended to spend less time “Standing” and more time “Walking” after treatment, none of the state behaviours were altered by the experimental treatments in any of the other groups. This may be because the treatments were not sufficiently noxious to change the stags’ normal pattern of behaviour or that these types of behaviours were not good indicators of discomfort about the pedicle. An alternative explanation could be that the stress of the situation of yarding, handling and holding in a pen for one hour masked any changes in expression of behaviours induced by applying the treatment. In contrast to other studies in deer (Pollard et al, 1993, 1994; Matthews et al, 1994) and calves (McMeekan et al 1999; Stafford et al, 2000; Theurer et al, 2012) the deer in the noxiousness study did not have access to feed or water. Feeding and rumination have varied between calves that were and were not dehorned (but our observations were for a short period of time (one hour) and it was felt that having food and water

in a small area may confound the other behaviours observed, especially if aggression occurred around the feeding area.

Standing Quiet

Most animals spent a large proportion of their time standing quietly with the mean and median both being approximately 50% of the time for both recording periods across all animals. The proportion of time spent standing quiet tended to decrease after 30 minutes in the pen and may indicate that the animals became more agitated and had a desire to move out of the pens after 30 minutes. This may be in response to hunger as they were not offered food during the penning or may simply be a natural fluctuation in the desire for movement by the animals. There was large variation in the proportion of time spent “Standing quiet” between animals and this most likely reflects the temperament of individual animals and may also be related to the social structure of the particular group of five animals.

Walking

On the whole deer did not spend a large proportion of time “Walking”. The mean was only about 6% and 8% of time for “pre” and “post” treatment recordings respectively. During “pre” treatment, 2 deer did not spend any time “Walking” and 3 deer did not spend any time “Walking” during the “post” treatment recordings. This is a little surprising as it would be expected that all the deer would have to “Walk” at some stage to get from one position to another. The proportion of time allocated to each behaviour would depend on the criteria used for each behaviour. It is possible that a different analyst may have graded more of the “Pacing” behaviour as “Walking”. Because all of the videos were analysed by a single technician there should be consistency between animals in the scoring so it is unlikely to affect the data analysis. Despite the uneven distribution of “Walking” data, there was a positive correlation between “pre” and “post” treatment “Walking” for individual deer. 67% of deer in the “Both” treatment group “increased” their frequency of “walking” during the “post” treatment period compared to the “pre” treatment period. This was not true for any of the other treatment groups and there is no plausible explanation for why this was the case in the “Both” group only. The frequency of “Walking” tended to increase slightly after 30 minutes of recording and this corresponded with a decrease in standing.

Pacing

After “Standing quiet”, “Pacing” was the second most common state occupying an average of 37 to 39% of the observation time. As discussed above, the scoring of the behaviours was somewhat dependant on the interpretation of the person analysing the tapes. It may have happened that a different observer would score more of the “Pacing” behaviour as “Walking”. Nevertheless, there was a single analyst throughout this experiment so the scoring is likely to be consistent within this experiment even if the results can not be directly compared to a similar experiment conducted by a different analyst elsewhere. All deer were scored as “pacing” at some time during each observation period but there was not a significant correlation between the “pre” and “post” treatment recordings for individual deer. There did not appear to be any significant effects of any of the treatments.

Deer in the *NaturO* group tended to “pace” less than deer in the other treatment groups both during the “pre” and “post” treatment periods. Over time the frequency of “Pacing” did not appear to vary to a great extent.

Walking Backwards

Graf and Senn (1999) reported a higher frequency of abnormal “backwards locomotion” in calves that were dehorned without local anaesthetic compared to those that were given local anaesthetic suggesting that this may be a behaviour demonstrated more in calves that experience pain around the head. There were no significant differences between treatment groups in the deer in our experiment. Approximately two thirds of deer “Walked backwards” at some time during the observation period. For the majority of animals this was a brief behaviour while one deer spent 24% of its time walking backwards. Overall, there was a tendency for deer that “walked backwards” during the “pre” treatment period to also “walk backwards” during the “post” treatment period indicating that this behaviour has some degree of repeatability for individual deer although the correlation was not strong or consistent in all treatment groups.

Lying down

“Lying down” was only demonstrated by a small number of deer. Eight deer “lay down” during the “pre” treatment period and 4 during the “post” treatment period. One deer spent 44% of its’ time “lying down”. There were insufficient numbers of deer demonstrating this behaviour to make analysis valid. It is possible, but unlikely that the behaviour is demonstrated by a deer that is relaxed or tired. As deer are generally considered flight animals, those unfamiliar with the species may not expect them to lie down in a stressful situation. Contrary to this, it is frequently observed by farmers and by the researcher that some deer do lie down during handling procedures including pregnancy diagnosis in a race. Wall (1979) observed that a deer in severe pain or distress, such as following non-lethal gunshot wounds, will isolate itself from its herd-mates and spend many hours lying down. Morrise et al (1995) also found no difference in the proportion of time spent lying down in calves following dehorning but more frequent getting up and lying down. In contrast, Pollard et al (1993) recorded that deer that were velveted spent less time “sitting” during the first two hours after velveting than those not velveted.

Head position behaviours

For the head position states “Head straight”, “Head left” and “Head right”, all deer demonstrated these behaviours, the data were approximately normally distributed and there was a positive correlation between the “pre” and “post” treatment recordings for individual deer indicating that head movements are repeatable for individual animals. For the positions “Head high” and “Head low”, the data were not normally distributed. Across all of the head position behaviours there were no significant differences in the proportion of time spent in each position or the number of times that deer demonstrated each head position. Head positioning did not appear to be affected by treat-

ment. Deer in the “Lignocaine” group had a relatively high proportion that halved the proportion of time with their heads “Head high” and “Head low”. As this was not the case in other treatment groups it is possible that the lignocaine had some effect on this behaviour. The anaesthesia and numbing caused by the lignocaine may have affected the tendency of the deer to want to move their heads up and down. Other studies recording turning the head to the left or right were not found but Pollard et al (1992, 1993, 1994) has recorded the period of time that deer spent with their heads at, above or below shoulder level with differences found between treatment groups.

Event behaviours

All of the event behaviours were not normally distributed and were skewed to the right such that the majority of deer demonstrated each behaviour only a small number of times or not at all, while a small number of animals demonstrated the behaviours at high frequency. Event behaviours were also less repeatable for individual deer than state behaviours with only “Nose to ground”, “Flick ears” and “Flick tongue” having a positive correlation between the “pre” and “post” treatment period for individual deer. There tended to be more differences between and within treatment groups for event behaviours compared to state behaviours which may be because these behaviours are more strongly influenced by the treatment or may reflect the more difficult data analysis.

Non-specific event behaviours

These behaviours were considered common behaviours of normal deer that may or may not be related to different stimuli and have been recorded by previous authors (Pollard et al, 1992, 1993, 1994; Webster and Matthews, 2006). In the current study, the behaviours were not predetermined but were recorded as they were seen. There are some behaviours recorded by other authors that were not included in this study, because they were not observed as independent behaviours during video analysis. For example Webster and Matthews (2006) recorded “Dropped ears” and “Yawn”. It is possible that different treatments may alter the frequency of these behaviours as this has been found in other studies but these behaviours did not differ between treatment groups in our study.

Nose to ground

Nose to ground was a common behaviour and was distinctive from “Head low”. 98% and 96% of deer demonstrated this behaviour at least once during the “pre” and “post” treatment periods Pollard et al (1992) found that deer nosed the ground frequently (average of up to 3.6 times/minute) and that there was a significant effect of different analgesic treatment on this behaviour. In our study, there was no significant differences within or between treatment groups.

Flick tongue

All deer “flicked their tongues” during both observation periods and this was not surprising as it is a frequently observed behaviour in deer and has been recorded in previous studies (Pollard et al, 1994; Webster and Matthews, 2006). In the study by Pollard et al (1994) velveted stags flicked their

tongues more than non velveted stags during the first hour after velveting and Webster and Matthews (2006) also found that velveted stags flicked their tongues more than non-velveted stags and that the frequency of this behaviour declined over 3-4 hours. Tongue flicking is often associated with aggression in stags and it may be demonstrated more often in aggressive or painful animals. There was a fairly strong correlation between the “pre” and “post” treatment frequency of “Flick tongue” for individual deer indicating that this is a repeatable behaviour related to the individual animal. Overall the frequency of “Flick tongue” was higher in the “post” treatment period than the “pre” treatment period with one deer in the “Lignocaine” group demonstrating “Flick tongue” 171 times during the “post” treatment period. This animal was an outlier. When the medians of all treatment groups during the “pre” and “post” treatment periods were compared by ANOVA they did not vary significantly, however when individual treatment groups were compared using Mann-Whitney sign ranked tests there were some differences. The *NaturO* and Control group both had a higher frequency of “Flick tongue” during the “post” treatment period. In the *NaturO* group there was also a net 30% of deer that “increased” the frequency of “Flick tongue” by double or more during the “post” treatment period compared to the “pre” treatment period. While this suggests that the presence of the *NaturO*TM ring may have increased the frequency of “Flick tongue”, the fact that it was also increased in the Control group suggests that it was more likely due to random statistical variation. It is unlikely that “Flick tongue” was related to the treatment type.

Flick ears

Deer have been shown to flick their ears more often if they were velveted compared to not velveted (Pollard et al, 1993, 1994) and with compression vs lignocaine analgesia (Webster and Matthews, 2006). In our study, deer demonstrated “Flick ears” at a lower frequency than expected (median approximately 4/hour) compared to higher frequencies recorded by the above authors. Deer in the “Lignocaine” group tended to “Flick ears” less often during the “post” treatment period. It is possible that the injection of lignocaine around the base of the pedicle caused a lack of sensation but not motor function of the ears and top of the head making the deer less sensitive to irritation around the ears. The presence of the *NaturO*TM ring on the pedicle in the *NaturO* and Both groups did not cause the deer to increase ear flicking behaviour. The frequency of “Flick ears” was quite variable over time and between treatment groups. It was observed that “Flick ears” was often associated with an external stimulus such as a fly in the pen and that all deer in the pen would be affected and would “Flick ears” in close succession. In order to further investigate this, the mean number of times deer demonstrated “Flick ears” on each film was reviewed. Table 3.4.17 shows a list of the tapes and the mean number of times deer “Flicked ears” on each of the tapes. Tapes 32 and 13 had a particularly high number of occasions of “Flick ears”. It is probable that the behaviour was affected more by external factors occurring during the particular recording period than by individual deer or treatment effects.

Shake

In the experiment by Pollard et al (1993), deer that were velveted shook their bodies and heads more than those that were not velveted and in a later study (Pollard et al, 1994) there was no difference between velveted and non-velveted stags in body shakes, but velveted stags did shake

their heads more than non-velveted stags. In our study, we did not observe “Head shakes” but most deer did “Shake” their body at some stage during the recording period (73 and 57% respectively) although the frequency of this behaviour was quite low, with the maximum being 6 times during 60 minutes in one deer. Overall, significantly fewer deer demonstrated “Shake” during the “post” treatment period compared to the “pre” treatment period however the difference in the median frequency of the behaviour was not significantly different overall. Within the *NaturO*TM ring treatment group there was a significant reduction in the frequency of “Shake” during the “post” treatment period compared to the “pre” treatment period and a net total of 43% of deer in this group “decreased” the frequency of “Shake” by at least 50%. The frequency of “Shake” was also lower during the “post” treatment period for the *NaturO* compared to all other treatment groups but not during the “pre” treatment period. Overall, it appears that the treatment with *NaturO*TM rings decreased the tendency for deer to “Shake” their bodies. This effect was only seen in the *NaturO* group and not in the Both group. The reason that deer in the *NaturO* group would be less inclined to “Shake” is not altogether clear but it is possible that the sensation of the *NaturO*TM ring would make the deer more cautious about vigorous body movements. If this was the case, it would be expected that the frequency of “Shake” would be lowest when the *NaturO*TM rings are most noticeable to the animal and other behaviours are also modified. This would be during the first 10 to 30 minutes as for the specific head related behaviours so it would be expected that the frequency of “Shake” would be lowest during this time and may increase thereafter. This was not the case however as the frequency of “Shake” was slightly higher during the first 10 minutes than at other times during the recording and this was true for both the “pre” and “post” treatment periods.

Table 3.5.1 Mean number of times that deer demonstrated the behaviour “Flick ears” during one hour of video observation for each video tape of a pen of 5 deer.

Tape	Mean frequency “Flick ears”
14	0.4
4	1
35	1
39	1
22	1.4
34	1.4
23	1.8
25	2
31	2.2
27	2.4
29	2.4
16	2.6
24	2.6
26	2.6
37	2.6
38	2.6
21	2.8
6	3
15	3.2
30	3.2
2	3.4
40	3.4
9	3.6
17	3.6
8	4.6
10	4.6
28	4.6
3	5.6
19	5.6
7	5.8
20	5.8
18	6.2
1	6.4
5	7.8
11	8.4
33	9.8
32	13
13	14.6
12	No data

Grooming

Self grooming was not a very common behaviour with less than 30% of the deer demonstrating this behaviour. Grooming other deer was demonstrated more than self grooming with 52% of deer demonstrating “groom other” during the “pre” treatment period. There was a significant overall reduction in the number of times that deer demonstrated “Groom other”. Within treatment groups there was significant reduction in the “Lignocaine” and “Both” groups. There is no easy explanation for why this would be the case. Perhaps the mutual grooming behaviour is triggered by some environmental or other stimulus that tended to be more prevalent during the “pre” treatment recordings that during the “post” treatment recordings. This study was conducted in late spring which coincides with the time of changing of coat from winter coat to summer coat and this may have influenced the “Groom other” behaviour. Other studies (Pollard et al, 1994; Webster and Matthews, 2006) have found an increase in grooming in velveted stags compared to non velveted stags. These authors have also recorded self-licking and licking other deer and it is not entirely clear what the differentiation is between grooming and licking.

Specific head related behaviours

These behaviours were not identified until after the start of viewing the video tapes as they have not been previously described in deer. They may be considered to be less common behaviours of deer and therefore may be more specific to the stimuli being applied in this experiment. Deer in the *NaturO* and Both groups had increases in most of these behaviours when compared to the “pre” treatment period. The effect of time on the frequency of these behaviours was also significant. During the “pre” treatment period there did not appear to be any obvious trends over time and most behaviours were seen at a low frequency throughout. During the “post” treatment period, all of the specific head related behaviours except for “Arch up” had a considerably higher frequency during the first 10 minutes of recording and dropped sharply during the next 10 to 20 minutes so that they were near the “pre” treatment levels by 20 to 30 minutes after the start of recording. Most of the rise in frequency during these first 10 minutes was due to an increase in deer in the *NaturO* and Both groups and is likely to be due to the presence of the *NaturO*TM ring. This effect did appear to subside fairly quickly though and may be due to either tolerance of the *NaturO* by the deer and thus a decrease in the frequency of the behaviour or may be due to the development of a lack of sensation to the *NaturO*TM ring as the ring itself may have provided analgesia consistent with the onset of lack of response to electrical stimulation of the antler (Nicol et al, 2009).

Head rotating left and right

Rotating the head was described as a rotation about the axis-atlantal joint whereby one antler was dropped down and the other raised up. For example “Rotate left” meant that the left antler was lowered. This was distinct from turning the head to the left during which the orientation of the head would remain upright and the deer would turn its neck to look to the left. Although a relatively large number of deer did demonstrate head rotating during the “pre” treatment period

with 23% rotating to the left and 25% rotating to the right, the number of deer that demonstrated these behaviours was significantly higher during the post treatment period with 38% rotating left and 41% rotating right. While this behaviour was not anticipated, the fact that about one quarter of the deer did show this behaviour suggests that it is occasionally displayed by untreated deer. It is possible that the presence of the Velcro caused some deer to rotate their heads. As there was not a group that did not have Velcro bands on this comparison could not be made as it is not known whether deer with no Velcro on their pedicles would rotate their heads. The median frequency of head rotating left and right showed a more marked increase than the number of deer that demonstrated the behaviour. The *NaturO* and the Both treatment groups significantly increased the frequency of rotating both left and right during the “post” treatment period. The change was greater in the *NaturO* group than the Both group but the difference between these groups was not statistically significant. A net 60% and 53% of deer in the *NaturO* group “increased” the frequency of “Rotate left” and “Rotate right” respectively.

Rotate head 360°

This behaviour was not anticipated before the start of the study as it had not previously been described and was not demonstrated at a high frequency, with only 4% of deer demonstrating “Rotate head 360 during the “pre” treatment period indicating it is not a normal behaviour of deer. One deer in the Lignocaine group had a relatively high frequency of “Turn head 360” during the “pre” treatment period but not during the “post” treatment period. The number of deer demonstrating the behaviour increased significantly to 16 and these deer were nearly all in the *NaturO* and Both treatment groups. This behaviour was quite specific for deer that had *NaturO*TM rings applied but was not very sensitive as there were a large number of deer that did not demonstrate this behaviour at all.

Arch head up and down

These behaviours were less specific than Rotate 360° although they did have significant increases during the “post” treatment period compared to the “pre” treatment period and those increases were due to significant increases in the *NaturO* and Both groups only. It appears that the presence of the *NaturO*TM ring did increase the frequency of these behaviours. It may have been difficult to distinguish head arching either up or down from other head positioning behaviours. “Arch up” was similar to but distinctive from “Look up” and “Head high” whereas “Arch down” had to be differentiated from “Head low” and “Nose to ground”. The main difference was that in the “Arching” behaviours there was flexion of the neck vertebrae so that the neck was in a curve rather than simply lifting or lowering the head. The “arching” behaviour was also not associated with other actions such as looking at, sniffing or licking something up high or down low, and were not associated with external stimuli. Head arching was fairly common in deer during the “pre” treatment period for all treatment groups with 46% and 39% of deer demonstrating “Arch down” and “Arch up” respectively. This suggests that these behaviours are not specifically related to the types of stimuli that were being applied by the treatment but that a *NaturO*TM ring does seem to increase the frequency of the behaviour. It may have been that deer were responding to the presence of the Velcro band during the “pre” treatment period. If this was the case it would be expected that deer

in groups 5 (Lignocaine both antlers) and possibly group 8 (Lignocaine and *NaturO*TM rings on both antlers) might have a decreased frequency of these behaviours. This is further explored below.

Head Twist

It was observed that different deer each have a different pattern of behaviour. While some deer might demonstrate “Rotate head” in response to the *NaturO*TM ring, others might demonstrate “Arch down” or “Press head”. None of these behaviours are likely to be universal indicators of the effect of the *NaturO*TM ring in their own right. These behaviours were therefore combined to make an overall “Head twist” score. By combining these behaviours it did increase the significance of the difference between “pre” and “post” treatment periods and also the difference between treatment groups in the “post” treatment period. The difference was not as great as might have been expected though and this is likely to be due to the non specificity of some of the behaviours, particularly “Arch down”. Overall there was an “increase” by at least 100% in “Head twist” for 80% of the deer in the *NaturO* group and for 56% of deer in the Both treatment group. No animals in the *NaturO* group showed a “decrease” in head twist. It is likely that the presence *NaturO*TM ring was responsible for this change in behaviour.

The effect of the Velcro band.

It was not expected that a Velcro band placed lightly on the pedicle of the antler would alter the behaviour of the deer to any great extent and that if it was perceived by the deer it would be less noxious than the potential effect of the *NaturO*TM ring. As discussed above, there was a tendency for deer to display some of the unanticipated behaviours such as head rotating and head arching during the “pre” treatment period as well as the “post” treatment period. One explanation for this might be that the Velcro band was enough of an irritant to the deer to cause some of them to demonstrate this behaviour. In order to investigate this, the data were reanalysed by combining the two treatment groups that had lignocaine anaesthetic applied in a ring block around both antlers (“Lignocaine both” and “Lignocaine both + *NaturO*TM ring both”) in addition to the Velcro band. Theoretically lignocaine could remove sensation that may be perceived by the presence of the Velcro band. The frequency of the “Head twist” was compared during the “pre” and “post” treatment periods for these two groups combined and the “difference” (post-pre) for each deer was also examined. This is shown in Figure 3.5.1. There was a significant decrease in the frequency of “Head Twist” for deer that had lignocaine applied to both antlers when animals that did and did not have a *NaturO*TM ring applied as well and the lignocaine ring block were included in the analysis (Wilcoxon signed ranked match-paired test, $p=0.0089$).

Both vs *NaturO* treatments

Deer in the Both treatment group had a *NaturO*TM ring on one antler and lignocaine ring block on the opposite antler. Across all of the specific head related behaviours except “Press head” both of these treatment groups had significant increases when the “post” treatment was compared to the

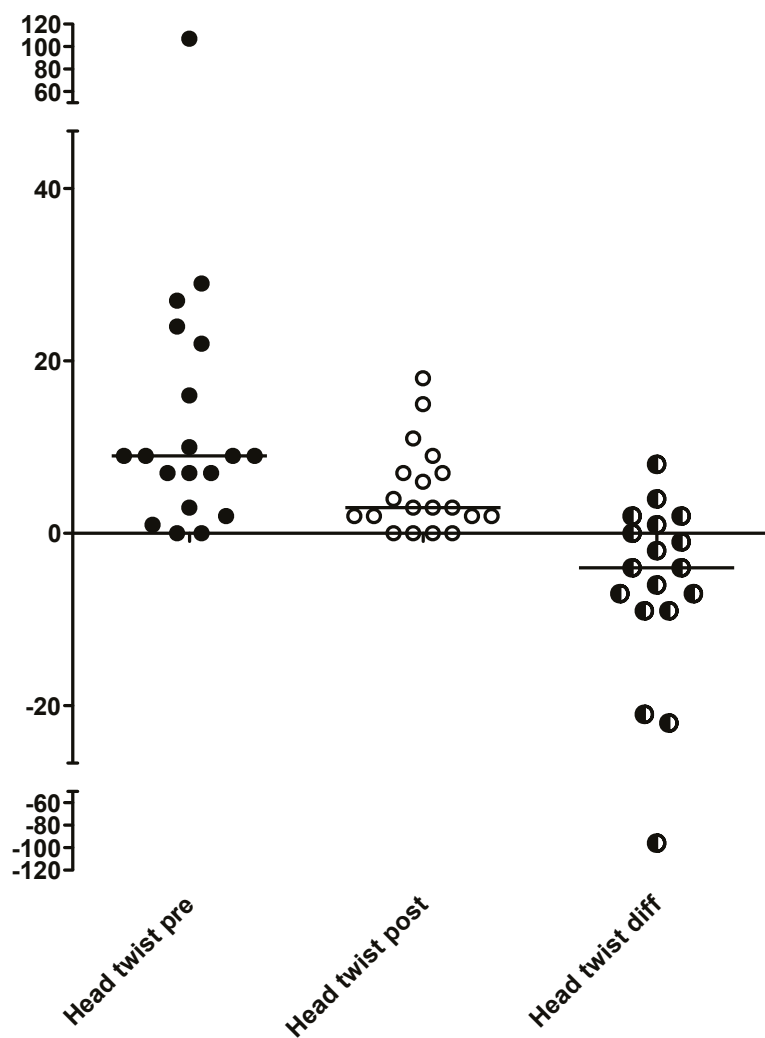


Figure 3.5.1 Number of times that individual yearling male deer demonstrated the combination of specific head related behaviour “Rotate left”, “Rotate right”, “Rotate head 360°”, “Arch down”, “Arch up” and “Press head” summated as “Head twist” during one hour of video observations. Deer were from two treatment groups that had a Velcro band around the pedicle during the “pre” treatment period and had a Velcro band and the addition of either a lignocaine ring block or NaturO™ rings and a lignocaine ring block applied to both antlers during the “post” treatment period. The diff is the difference (post-pre) in frequency for individual deer

“pre” treatment recording but the overall effect was less in the Both group than the *NaturO* group. This might be because the lignocaine that was applied around the opposite antler was having a confounding effect for animals in the Both group. The dual effect of numbing on one antler and *NaturO*TM ring on the other may have altered the response of the deer or the lignocaine applied in a ring around the base of one pedicle may have offered some local anaesthesia to the antler that had the *NaturO*TM ring applied.

Statistical analysis of behaviour data

Statistical analysis of the data in Chapter 3.4 was challenging and limited in its power because of the nature and spread of the data. For some behaviours that had a high frequency in a large proportion of the deer comparisons between groups were straightforward as the data was approximately normally distributed so standard tests comparing the means could be performed. For many of the behaviours of interest, the data were not normally distributed and were skewed to the right. In the first instance it was considered that a logarithmic transformation may convert the data to a normal distribution however this was hindered by the large proportion of deer that did not show the behaviour at all and hence scored zero. For this reason, non parametric tests were done. To compare two medians, the Wilcoxin signed ranked test was performed instead of the t-test. As pre and post treatment values were available for all animals, this test was able to take into account repeated measures. To account for the zeros a transformation can be done that adds 1 to all the results of count outcomes. However this still does not create a normal distribution although in most cases non-zero values will become normally distributed and overall the data usually passed a normality test. The power of the normality test is questionable in this situation. The question was whether this data manipulation in order to be able to perform a statistical test for comparison of means would add extra value to the interpretation of the results. In this type of data analysis and interpretation a relatively high emphasis should be placed on biological interpretation and plausibility along with some judgement on the implications of the results. There are no statistical tests that can determine the relevance of any particular behaviour to the welfare of the animal or the degree of pain experienced. Similarly, no statistical tests can determine the acceptable number of animals that should change their behaviour in response to a treatment. If one single animal out of the group of 10 showed an adverse reaction to a stimulus this would not be statistically different from zero animals in the group showing a reaction. However 1 out of 10 may be an unacceptable number of animals that show a response to noxious stimulus. The judgement of the acceptable level and the interpretation of the significance of differences between groups comes down to the individual person dealing with the animals and the values that society and those in control of protecting the rights and welfare of animals hold. For these reasons it was considered more useful to present the data in as close to the state it was collected as possible. So for variables that had a large number of zero outcomes and a wide range in frequency for those animals that did demonstrate the behaviour, these are presented as such wherever possible. In this way, the reader can get an impression of the proportion of animals that changed their behaviour in each group and the magnitude of that change for individual animals. Non-parametric (sign ranked) statistical analyses were used on raw data. If the data were transformed to approximate a normal distribution, the statistical tests may have had greater power and more capable of detecting a statistical difference between treatment groups.

EEG studies

EEG recording under general anaesthesia was chosen for this study to provide an objective measure of a response to a noxious stimulus while removing the influence of external stressors or stimuli that may make behavioural and physiological measures variable and difficult to interpret. This method allowed inclusion of a control group without compromising the welfare of the animal (Murrell and Johnson, 2006; Murrell et al, 2003). Halothane was used as the inhalation agent as it does not suppress the EEG as much as other inhalation anaesthetics (Leduc, 2006; Murrell et al, 2008), has no analgesic properties and previous studies have found that animals anaesthetised with halothane show greater changes in F95 in response to anaesthetic depth and possibly noxious stimulation than with other inhalation anaesthetic agents (Llyod-Thomas et al, 1992; Dwyer et al, 1994; Johnson and Taylor, 1998; Antunes et al, 2003a).

End tidal halothane was used to standardise anaesthetic depth between animals. In this study, ET halothane was maintained at $0.91 \pm 0.9\%$. This is slightly greater than the 0.8% maintained in a previous study in deer (Johnson et al, 2005) and was chosen so that movement response to antler cutting that may contaminate the raw EEG would be minimised. Most animals demonstrated a large spike in the EEG during antler cutting and this time period was removed from analysis. It is not known how long it would take for the EEG wave-pattern to return to normal after cutting but presumably this would be quicker at a deeper plane of anaesthesia. It is unclear whether the typical changes seen in response to a noxious stimuli are a result of the specific afferent nerve input signals or a more general waking from anaesthesia and increased cerebral activity when a noxious stimuli is applied. Increases in F50 and F95 and decrease in TP in response to noxious stimulus may be more pronounced at this depth of anaesthesia but that is yet to be tested in deer. Horses have shown greater trend towards high amplitude, low frequency EEG activity at a deeper plane of halothane anaesthesia (Johnson and Taylor, 1998) which may allow greater scope for a detectable response but a deeper plane of anaesthesia has generally resulted in a reduced EEG response to acute somatic noxious stimulus (Antognini and Carstens, 1999; Orth et al, 2005, 2006). Only ten out of sixteen stags in the control group had a significant response to antler cutting. It is possible that the lack of response was due to an inappropriate plane of anaesthesia but a more likely explanation is individual animal variation in both the unconscious and conscious EEG patterns.

The induction agents chosen were propofol and ketamine. These have been shown to produce rapid induction of anaesthesia to a plane to allow intubation of deer (Johnson et al, 2005) and have a short duration of effects on the CNS and EEG. The effects of different anaesthetic agents on EEG is not clearly understood but a few experiments have shown that both drugs are likely to affect the EEG and change the response pattern to noxious stimulus over a relatively short period of time after administration in other species. Ketamine, a dissociative anaesthetic with strong analgesic properties, caused a reduction in F50 in horses maintained on halothane anaesthesia that returned to baseline within 15 minutes of infusion (Johnson et al, 1999). Cats given an intramuscular bolus of ketamine demonstrated obliteration of EEG response to noxious stimulus

that lasted up to 24 minutes (Taylor and Vierck, 2003). Rats maintained on propofol did not show dose related changes in either F95 or F50 (Antunes et al, 2003). There is no PK PD data for EEG effects in any species. In the current study, baseline recordings did not commence until 30 minutes after induction of anaesthesia and the experimental data collection began 60 minutes after induction allowing time for the drugs to be metabolised. The clear response seen in some control animals suggests that this was long enough. Although the lack of response in some animals is unlikely to be due to residual induction agents, this cannot be completely ruled out as there will be some between animal variation.

Anaesthesia procedure

Catheterisation of a vein of the face was successful in all cases. In some cases the catheter was inserted into the facial vein and in other cases into a nasal branch towards the nostrils. This depended on the personal preference of the person doing the catheterisation. In the author's opinion the more nasal location was easier because the vein was more superficial and tended to run in a straight line whereas the facial vein tended to curve ventrally and ran around the angle of the mandible. There was no need to raise the vein as the pressure from the crush was enough to keep the vein raised. There were valves irregularly distributed along the veins and these were easily identified and could generally be avoided by choice of catheter entry point. An alternative site for catheterisation may be the ear vein. It may be more difficult to catheterise this site due to animal movement. Other sites were not accessible while the animal was restrained in the crush.

A bolus dose of 5mL ketamine and 40mL propofol was administered as rapidly as possible through the 20 gauge catheter. The range in dose rates was 4.5 to 7.1 mg/kg of ketamine and 4.8 to 16.0mg/kg of propofol. The difference in ketamine dose was due entirely to the range in bodyweights (70 to 112 kg) and the variation in propofol dose was due to differences in bodyweight and in top-ups required before intubation was achieved and anaesthesia was able to maintained with inhalational anaesthetic agents. There is no suggestion that this difference in induction dose rate had an effect on EEG pattern. Within the control group there was no effect of dose rate on the presence of an EEG response after cutting the antler.

Induction was rapid and smooth in all cases although a top-up dose of propofol was often required, particularly with a difficult intubation that took more than 2 minutes. Deer often went into apnoea after induction although this was very difficult to assess with the animal restrained in the crush and a person performing intubation. Another person was required to hold the larynx which obscured the deer from view somewhat. Better monitoring may have been possible if the animal was dropped to the floor and intubated in recumbency. The endotracheal tube was placed by manually advancing the whole hand and end of the tube through the oropharynx and manipulating the tube over the epiglottis and between the arytenoid cartilages. The width between the back teeth varied between deer and in animals with a small space, the sharp teeth would graze the sides of the operators hands. A person with small hands was required to intubate these smaller deer.

Initial stabilisation of inhalation anaesthesia was tricky because apnoea or irregular breathing patterns often occurred after induction and a variable period of time elapsed before spontane-

ously regular breathing was seen. This resulted in lightening of the plane of anaesthesia during periods of apnoea which was often followed by fast shallow respiration causing a rapid deepening of anaesthesia and then another period of apnoea. After several minutes, most deer established a regular respiratory pattern but some deer did not develop a regular respiratory pattern throughout the entire anaesthetic period. Erratic anaesthetic depth could be prevented by intermittent positive pressure ventilation. While this helped to maintain anaesthetic depth, it also tended to delay spontaneous respiration, presumably by maintaining a low carbon dioxide level and thus reducing the drive to breathe. This could possibly affect the EEG, but variable depth of anaesthesia would have a much greater effect.

In all cases, anaesthesia was stable at the time of antler removal and this was a requirement of the experimental protocol. Anaesthetic depth was largely judged on expired end tidal halothane concentration.

Baseline recordings

Baseline recordings were seen to be fairly stable over time for F50 and F95 and less so for TP, with some animals having a more stable baseline than others. Channel one and channel two from the same animal correlated well in most cases except the magnitude of TP was often quite different. There was variation between animals in the absolute values of the baseline measurement for F50, F95 and TP. Thus converting each value at each time point to a percent of the baseline was a valid method to allow comparison between treatments. F50 and F95 represent the frequency of change of current direction between the electrodes whereas TP represents the magnitude of the recorded signal. TP will be influenced by the conductance of the recording equipment and it could be expected that the electrodes and wires on either side on the animal would have slightly different conducting properties. That would explain these differences. The pattern of change in TP over time was similar between channels.

Effect of antler removal without analgesia on the EEG

Analysis of the average EEG power spectrum of control animals (Experiment 3.1) showed a general pattern of decreased TP, increased F50 and increased F95 in response to antler cutting. This response is sometimes referred to as de-synchronisation (Murrell et al, 2006). These results are similar to those found during antler removal in adult stags (Johnson et al, 2005) and immediately after scoop dehorning calves without analgesia (Gibson et al, 2007). A decrease in TP and increase in F50 but not F95 was shown in response to electrical and thermal stimulation but not mechanical stimulation to the tail of rats (Murrell et al, 2007a) and in horses in response to castration (Murrell et al, 2003) under a similar anaesthetic protocol to the one used in this study.

Figure 3.5.2 shows the time series of TP, F50 and F95 for each second from five minutes prior to treatment until ten minutes after treatment for a typical EEG response to antler cutting without analgesia. Figure 3.5.3 shows a typical spectral array for a deer in the control group. Time is plotted on the x axis from left to right, power is plotted on the y-axis and on the z-axis is each frequency band from 30 Hz at the front of the graph and 1 Hz at the rear of the graph. In this

figure it can be seen that there is a large drop in amplitude at the rear section (low frequency) of the graph for about one and a half minutes after antler cutting (indicated by the thick white line). There is a small increase in activity in the higher end of the spectrum towards the front of the graph, particularly in the very high bands but the relative changes here are variable.

It is likely that the decrease in total power seen in the control group is due to a reduction in power in the lower end of the frequency spectrum. There may also be a corresponding increase in power in the higher end of the spectrum but this is relatively a lot less as there tends to be a negative correlation between frequency and amplitude. F50 and F95 may be increased because of a higher level of activity in the high frequency end of the spectrum and/or a lower level of activity in the lower end of the frequency spectrum. The latter is the more likely explanation and is demonstrated in figure 3.1.9 which shows a marked drop in activity at the low end of the frequency spectrum and only a relatively small change at the upper end of the spectrum.

Murrell et al (2003) suggested that F95 may be a more specific indicator of anaesthetic depth while F50 may be more responsive to acute noxious stimulus. In most studies examining the EEG response to noxious stimulus, changes have been seen in F50 but not F95.

In our experiments, significant changes in F95 were seen both in response to antler cutting without analgesia and in response to lignocaine injection but the changes were in different directions. It is possible that the increase in F95 in response to antler cutting was a reflection of a lightening plane of anaesthesia rather than pain *per se* and that the decrease seen in F95 after lignocaine injection reflected a central depressant effect on the brain. We found that small changes in F95 were highly significant and this finding was similar to that of Gibson et al (2007) following scoop

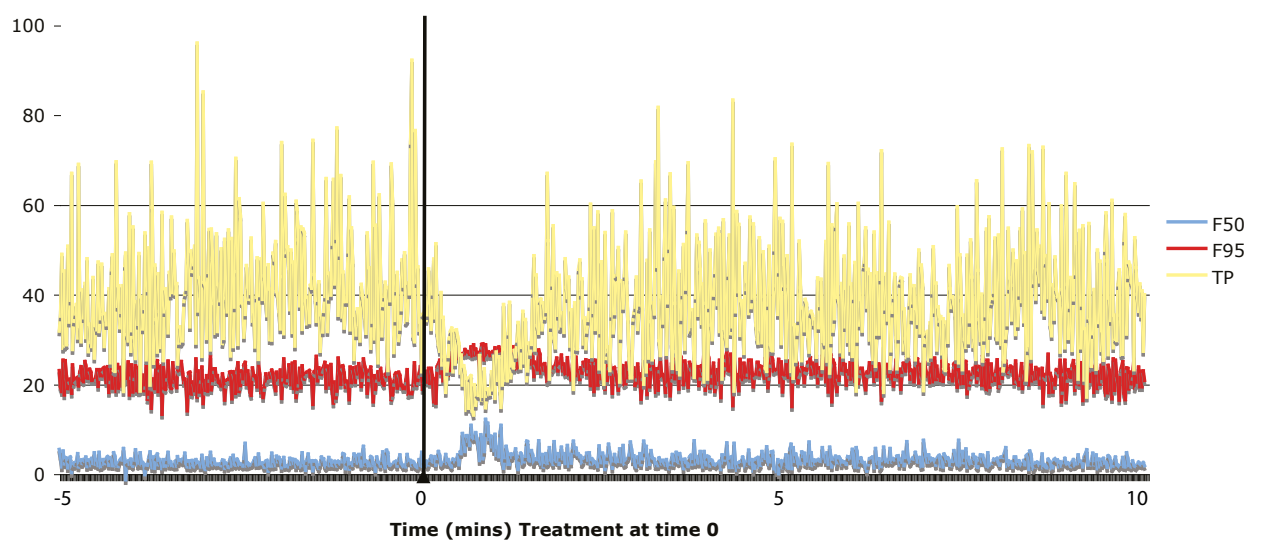


Figure 3.5.2 Total Power, Median Frequency and Spectral edge frequency recorded from a single channel of EEG in an anaesthetised rising one-year-old male deer that was being treated with a NaturO™ ring. The figure shows actual one second values from five minutes prior to treatment until ten minutes after treatment. Time of treatment is indicated by the black line.

dehorning of calves.

Effects of a lignocaine ring block or NaturO™ ring on the EEG response to velvet antler removal

Applying lignocaine in a ring block around the base of the pedicle at least 5 minutes prior to cutting the antler eliminated the EEG response that was seen during and after antler cutting compared to the control group. This is similar to the EEG response to scoop dehorning of calves following local anaesthetic application (Gibson et al, 2007) and velvet antler removal in deer following local anaesthesia (Johnson et al, 2005). There was a significant increase in Total Power immediately after cutting the antler and this is likely to be due to movement noise from antler cutting. It was not related to any changes in F50 or F95. There were few changes in F50 and F95 after cutting the antler and any that were present were relatively small and did not follow any pattern with time. This finding supports that assumption that changes in the EEG seen in the other groups were due to cutting the antler while the sensory nerves remained functional. Another possibility that can not be ruled out is that lignocaine has a direct effect on the brain and affects the EEG pattern centrally rather than solely by preventing conduction in axons as discussed below. It is not known to what extent this central action would alter the EEG pattern in response to nox-

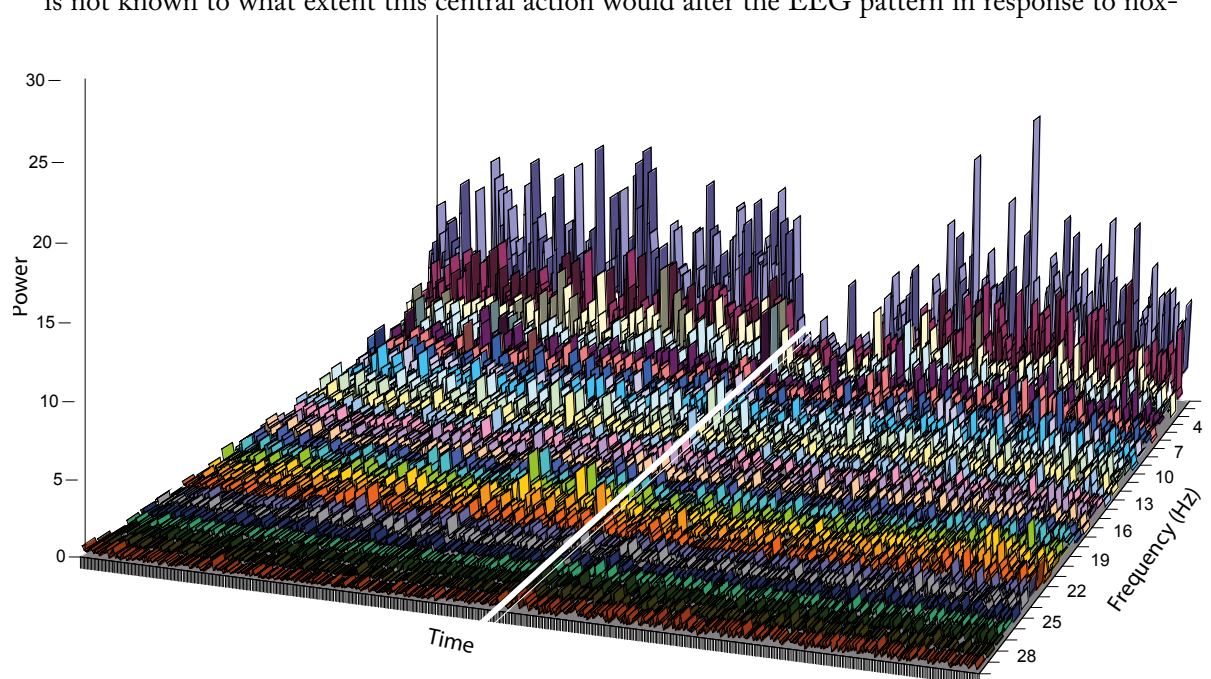


Figure 3.5.3 Frequency-Power-Time spectrum of the fast-fourier transformed EEG waveform in a single deer during velvet antler removal under general anaesthesia. The deer was not given any analgesia prior to cutting the antler.

Across the front axis (x axis) is time in one second epochs from 5 minutes prior to antler cutting to 5 minutes post antler cutting. The time of antler cutting is indicated by the thick white line. On the z-axis are the frequency bands in 1Hz groups from 30 Hz (high frequency) and the front to 1 Hz (Low frequency) at the rear of the graph. The vertical (y) axis shows the power generated in each frequency band at each time period.

ious stimulus. This could be tested by injecting lignocaine into a vein prior to measuring the EEG response to cutting the antler that was not provided with local anaesthetic. As a central effect was not anticipated in the current study, this was not done.

The *NaturO*TM ring appeared to attenuate the EEG response to antler cutting compared to the control group but not to the same degree as the lignocaine group. In the *NaturO*TM ring group, the EEG parameters, TP, F50 and F95 were altered in response to antler cutting to differing degrees compared to the control group with minimal changes in TP, moderate significant increases in F95 and clear significant increases in F50. Although there were significant changes from baseline, there were few statistically significant differences between the *NaturO*TM ring group and either the lignocaine or the control group. The results of these analyses were averages within each treatment group and did not take into account the proportion of animals that did or did not respond to antler cutting after treatment. It is doubtful whether the magnitude of the average change for the *NaturO*TM ring group can be extrapolated to individual animals as fewer animals in this group had a significant change to antler cutting than in the control group, so the average may simply reflect this fact.

Effect of application of lignocaine on EEG

Lignocaine caused an increase in total power, and a decrease in spectral edge frequency and increase in median frequency. These changes took several minutes to reach maximum levels. While increase in total power and decrease in spectral edge frequency are often seen with increasing plane of anaesthesia, an increase in median frequency is not consistent with this. A deeper plane of anaesthesia would result in increased activity in the low frequency range which would cause a decrease in F50. It took about two minutes for effects to be seen and the magnitude of these changes increased over about five minutes after which time they stabilised. This could be consistent with lignocaine transfer from the site of application into the blood-stream and being transported to the central nervous system, having its effects on the EEG at that location. Lignocaine may cause increased activity in one part of the frequency spectrum. Figure 3.5.4 is a spectral array for Deer 3, channel 2, showing the power generated in each of the frequency bands from 1 to 30 hertz during each second of recording. The white line indicates time of treatment with a lignocaine ring block. This graph shows an increase in activity in the frequency range of 18 to 25 Hz that becomes most evident about three minutes after treatment and persists until the end of recording. This coincides with a marked decrease in activity in the highest frequency bands (26 to 30Hz).

Murrell et al (2005) administered a constant intravenous infusion of lidocaine to ponies during anaesthesia for castration and recorded the EEG. Compared to an earlier study in which ponies were castrated under general anaesthesia without lidocaine infusion (Murrell et al, 2003), the lidocaine attenuated the EEG changes seen in response to castration. In the second study (Murrell et al, 2005) the EEG results from the first and second testicle removals were compared. Although there was no significant difference in the spectral edge frequency between testicle one and testicle two, the spectral edge frequency was significantly lower than baseline during the removal of testicle two but not testicle one. It was also noted that the serum lidocaine levels were higher at the conclusion of the removal of testicle two compared to the start of testicle one. The authors suggest

that the spectral edge frequency may represent the hypnotic effects of anaesthetic agents and be selectively sensitive to different drugs.

Effects of application of NaturOTM rings on EEG

NaturOTM rings caused an increase in F50 and an increase in F95 that was first seen as a significant change from baseline during the first minute and remained elevated ($p < 0.01$) until eight minutes after treatment. There was also a decrease in TP that was only significant during the second minute. Increase in F50 and F95 indicate a relative increased activity in the high frequency range and/or a relative decrease in the low frequency range. When this is seen along with a decrease in total power, it may indicate increased state of arousal. This pattern has been seen in response to somatosensory noxious stimulus in anaesthetised deer (Murrell and Johnson, 2006). Figure 3.3.6 shows the TP, F50 and F95 as actual output from the FFT of the raw EEG in deer 18 channel 1 and shows increase in F50 and F95 and concurrent decrease in TP during the first one to two minutes after treatment with a NaturOTM ring (black arrow). This is similar to the response seen when a noxious stimulus has been applied in other experiments.

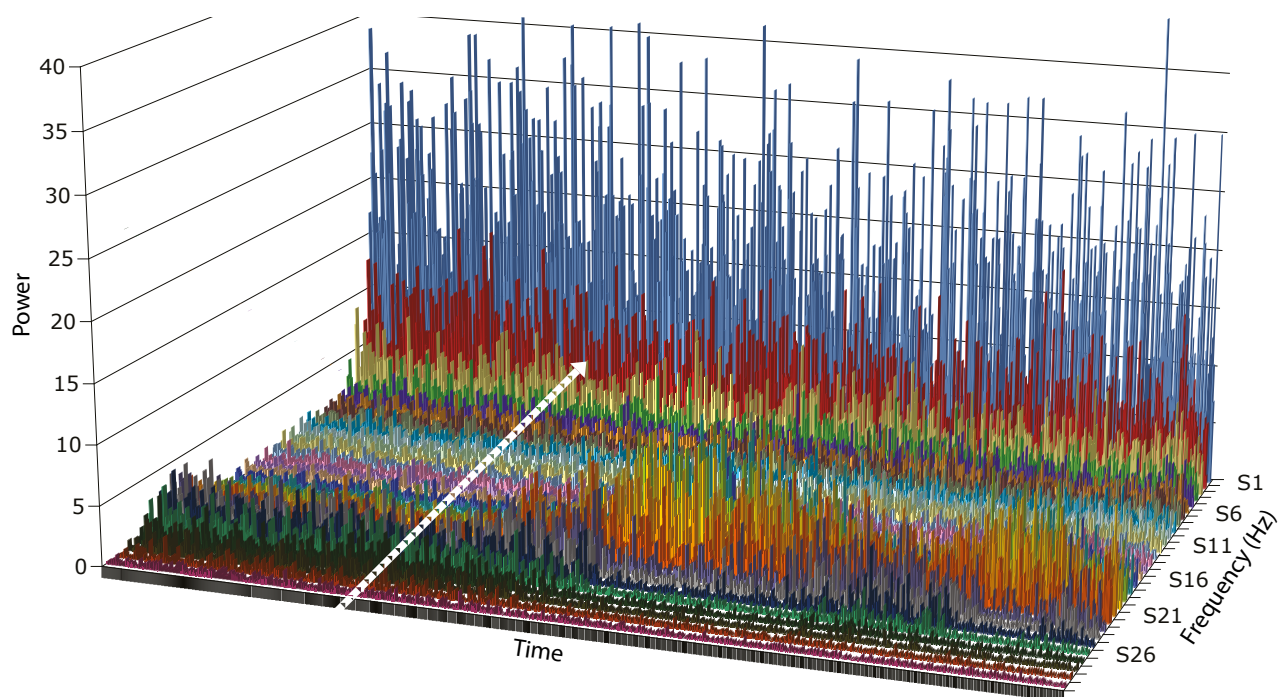


Figure 3.5.4 Full spectral array for the fast-fourier transformed electroencephalogram from a rising one-year-old male deer (Deer 3, channel 2) that was administered a lignocaine ring block around the base of the pedicle at time zero. Time is shown on the x axis from 5 minutes before treatment until 10 minutes after treatment. Treatment is marked with the white arrow. Frequency is shown on the z axis with low frequency (1Hz) at the rear of the graph and high frequency (30Hz) at the front of the graph and power is shown on the y axis.

The time course of changes seen in the *NaturO* group was different to that seen in the lignocaine group. Significant effects were evident earlier after *NaturO* treatment and did not persist as long as those seen after lignocaine. There are several possible explanations for this. One is that lignocaine causes a prolonged alteration of the baseline EEG recording that last at least 10 minutes whereas the acute noxious stimulus of high pressure compression may only cause alterations that are short-lived before negative feedback or gating occurs. Alternatively, the *NaturO*TM ring may provide analgesia that is rapid in onset and therefore reverses the changes seen after application. This is unlikely however as the industry standard is to leave the *NaturO*TM ring on for one hour before analgesia is expected to be fully achieved in all animals. Another possibility is that if noxious stimulation causes increased arousal and lightened plane of anaesthesia, it may increase the respiratory rate of the deer and thus inhaled halothane volume. This would cause the animal to then increase its anaesthetic depth as the deer were breathing unassisted during recording.

EEG non-responders

EEG response to noxious stimulus varied between animals and not all animals that had antlers removed without analgesia demonstrated a significant change in EEG. It may have been appropriate to test each animal to see if it has a quantifiable EEG response before including it in the study. Johnson et al (2005) tested the antlers of 2-year-old deer before including them in a similar EEG study.

Table 3.5.2 shows the number of animals that showed an EEG response to cutting the antler in each treatment group in experiment 3.1.

Chi squared analysis of the data in Table 3.5.2 showed that the proportion of animals that showed an EEG response in the control group was significantly greater than those in the lignocaine group ($p=0.0001$). The proportion of responders in the control was not significantly different from *NaturO*TM ring ($p=0.077$) and the proportion of animals that showed a response in the lignocaine group was significantly less than those in the *NaturO*TM ring group ($p=0.015$).

Ten out of 16 deer in the control group showed a response to antler cutting. This suggests that approximately 2/3 of animals in the other groups should show a response to the same stimulus if no analgesia had been applied. Half this number (5/16) responded in the *NaturO* group and none of the animals in the lignocaine group. This would suggest that some animals have the pathway disrupted when a *NaturO*TM ring is applied, and half of the animals still have cerebral pain processing when the antler is cut after one hour of *NaturO*TM ring application. The difference between control animals and *NaturO*TM ring animals approached significance but the small sample size and the zero result in the Lignocaine group reduced the validity of this type of analysis.

The fact that not all animals were EEG responders in the control group did not preclude analysis of the results as determining the mean of each group took account of this. The average response of deer in the *NaturO*TM ring group was less than that of deer in the control group. This was largely due to the fact that only half as many deer responded in the *NaturO*TM ring group as in the Control group which affected the average. It may in fact be more appropriate to analyse the data in terms

Table 3.5.2 Number of animals that showed an EEG response to cutting the antler after treatment with either a NaturO™ ring, lignocaine ring block or no treatment (control). Superscript letters indicate significant differences between treatment groups.

Treatment	EEG response	No response	Total
Control	10 ^a	6	16
NaturO™ ring	5 ^b	11	16
Lignocaine	0 ^{ab}	16	16

of the proportion of responders rather than trying to determine a difference in the magnitude of the response. There is large variation between animals in the magnitude of the changes after antler cutting and this is unlikely to be well correlated to the amount of pain induced. Response to noxious stimulus may be all-or-nothing. This theory has not been tested because different levels of noxious stimulus would have to be applied in the same animal and the percent change recorded for each level of stimulus. The study was not designed to determine the difference in the proportion of responders in each group and it would have required much greater numbers of animals per treatment to have a reasonable level of certainty of the results.

Data analysis for chapter 3.3 included the proportion of deer that showed a significant increase or decrease in each of the parameters (TP, F50, F95). Depending on the interpretation of the data, between four and eight of the ten animals treated with lignocaine and five out of ten animals treated with a NaturO™ ring could be considered to have demonstrated an EEG response to treatment. Two animals out of ten given lignocaine did not show an EEG response to this treatment.

The proportion of animals that showed a clear EEG response to application of either lignocaine or a NaturO™ ring was approximately the same whereas the proportion of animals that clearly showed no response was higher in the NaturO™ ring group (5/10) than in the lignocaine group (2/10). It appears from these data that a more consistent response in EEG will be shown after administration of lignocaine than after administration of a NaturO™ ring.

While approximately half of the animals showed an EEG response to application of the NaturO™ ring, approximately 2/3 showed a response to velvet antler cutting without analgesia in experiment 3.1. The small sample sizes meant that this difference was not statistically significant.

The mechanism by which the NaturO™ ring mitigates the response is not clear, but is relevant to the welfare of the stag. This study indicates that complete blockade is not achieved in all deer one hour after application of the NaturO™ ring. One possible explanation could be incomplete ischaemia. In that case, small diameter or non-myelinated type C nerves that are able to withstand compression but not ischaemia may be able to continue to conduct slow action potentials (Fern and Harrison, 2004a,b). Larger diameter, myelinated type A nerves that are less resistant to direct compression may be prevented from transmitting fast action potentials. Blockade of the initial sharp pain sensation that would cause a flinch response may be achieved, while sensation commencing a few seconds after the initial insult as a consequence of persistent tissue damage may still be propagated. This theory would make behavioural observations misleading as these usually

focus on the immediate withdrawal response during cutting and not any sensation that might occur in the seconds or minutes following cutting. Response to electrical stimulation of the antler would also be expected to be absent as again, the response is seen as an acute withdrawal behaviour at the time of electrical stimulation. This may also explain the discrepancy between the behaviour (3.2) and EEG (3.1) studies as the slower onset of activity may still be detected by EEG.

Histological examination and action potential conduction studies may help to determine the mechanism of proposed analgesic effects of the *NaturO*TM ring. Behavioural analysis (Chapter 3.2) took place at the same time as the EEG study on different animals. Because the behavioural observations were taken before the results of this EEG study were known, the focus was on immediate withdrawal response at the time of cutting. It may have been more useful to record the behaviour of stags for a longer period following antler cutting to look for any slow-conducting pain related behaviours similar to observations made by Webster and Matthews (2006) following velvet removal with high pressure compression bands.

The results from these experiments indicate that application of a *NaturO*TM ring one hour prior to velvet antler removal prevents some responses to antler removal in some deer but not all. Most normal behaviours of deer are not affected by application of a *NaturO*TM ring but some deer showed abnormal behaviours at high frequencies. The methods used in these experiments were appropriate but improvements in experimental design would have improved to interpretation of the data, especially with respect to the behaviour experiments. In all cases, it would have been preferably to use of larger number of animals in each treatment group. The animals should be from the same source farm and adapted to the experimental procedures. The use of video recording was valuable but the time of video observation should have been longer in experiment 3.1 and injection with a long acting tranquilliser may have helped with interpretation of the behavioural response to velvet antler cutting. The results from this study concur with those of Nicol et al (2009) and suggest that a *NaturO*TM ring is better than nothing for providing analgesia for velvet antler removal but not as good as a correctly placed lignocaine ring block.

Chapter 4

Antler blood flow, lignocaine residues and tourniquets

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Introduction

Lignocaine hydrochloride is a commonly used and effective local anaesthetic for velvet removal (Wilson et al, 1999, 2000; Bartels et al, 2001; Woodbury et al, 2001, 2002, 2005). The issues relating to lignocaine use and lignocaine residues are introduced in Chapter 1.4. Residues of lignocaine have been found in velvet antler destined for sale and human consumption (Clear and Morris, 2005; Bagonluri et al, 2005) and this poses a potential food safety issue (International Agency for Research on Cancer). It is hypothesised that lignocaine enters the antler via the blood stream after injection below the level of the tourniquet. The deer industry would like to develop a method of preventing noncompliant residues (Morris and Clear, 2005) based on a tourniquet that reliably prevents arterial supply to the antler and thus prevents lignocaine from entering the antler. In order to develop a tourniquet for this purpose, it was necessary to investigate the arterial supply to the antler and the effects of different tourniquets on this. There are limited studies describing the blood supply to the antler (Adams, 1979; Suttie et al, 1985). Injection of methyl methacrylate (Murakami, 1975) into the carotid arteries can provide a cast of the arterial supply, including small vessels that may not be observable using fresh dissections. The tissues can then be eroded using desmesid beetles (Hefti et al, 1980), resulting in a relatively undisturbed model of the arteries. Measuring the blood flow in the intact antler is challenging and may be affected by anaesthesia. A series of experiments were conducted using three different methods including fluorescent microspheres (Chapter 1.4), cinefluoroscopy/angiography (Suttie et al, 1985) and direct observations of haemorrhage while a tourniquet was applied at different tensions. Once the tension required to prevent antler blood flow had been established, it was necessary to develop a tool that could be used by farmers in the field that would ensure compliance with lignocaine residues. There are a wide range of tourniquet materials in use (personal observation during data collection for Chapter 2) with farmers having personal preferences for certain materials and methods. There are no data that relate the type of tourniquet used with the residue level in velvet antler. For a standard tourniquet system to be made compulsory, it should be acceptable to farmers and the animals but also provide consistently low residue levels in antler. In the final experiments in this chapter lignocaine residues were measured from antlers that were removed following application of different tourniquets.

4.1 Fluorescent microspheres to determine the effect of tourniquet tension on antler blood flow in adult red deer

Introduction

This experiment used fluorescent microspheres (Chapter 1.4.3) to estimate relative blood flow to the antler of stags with tourniquets of different tension applied to the antler pedicle. It was hypothesised that a tourniquet applied around the pedicle of the antler would prevent fluorescent microspheres from entering the antler of the live deer.

Materials and methods

Animals

Eight stags, at least three years old, were selected for this study. These plus one other used for a pilot trial were purchased from a commercial farm in the Manawatu at the time of antler button drop (August) and managed on the Massey University deer unit until velvet was at the optimum length for commercial harvest (October). Mature stags were selected because it was believed that tourniquet failure or leakage is more likely in a mature stag than a young stag. The stags used are shown in Figure 4.1.1. Stag tag number, bodyweight, pedicle diameter, antler weight and experiment date are listed in Table 4.1.1

Treatments and Controls

Six treatments were given to each animal corresponding to six tourniquet tensions and six different coloured spheres. Each animal acted as its own control in two ways:

- The tourniquet was applied to one antler only and the other acted as a control for all colours of spheres.



Figure 4.1.1 Photograph of the eight mature stags used in a study using fluorescent microspheres to determine the relative blood flow to the antlers with tourniquets of different tension applied to the antler pedicle.

Table 4.1.1 Details of stags used for fluorescent microsphere study including bodyweight (where recorded, pedicle circumference, antler weights, estimated and actual velvet harvest dates.

Tag	Weight	Pedicle (mm) (Left/Right)	Antler weight (Left/Right) kg	Est. Harvest date	Comments and actual experi- ment date
1825		154/154	1.59/1.95	16 Oct (Sun)	13/10/05 (Pilot)
715	251	168/167	2.29/2.89		17 Oct 05
#3	163	196/196	1.83/2.72		20 Oct 05
1822		170/169		16 Oct (Sun)	21/10/05
1827		174/174	1.74/2.25	25 Oct (Tues)	26/10/05
1823	176.5	201/182		16 Oct (Sun)	27/10/05
1824	178	182/180		28 Oct (Fri)	27/10/05
1818	175	169/169	1.56/2.00	1 Nov (Tues)	28/10/05
1083	182	149/150	1.66/1.74	25 Oct (Tues)	31/10/05
1819				5 Nov	Not used
1820				8 Nov	Control, no microspheres injected.
1052				15Nov +	Pacing, move to spikers?
1826				16 Oct (Sun)	Not used

- The first injection (red) was given with no tourniquet so acted as the control colour to that antler.

One animal (1820) acted as a control. The anaesthetic and antler removal procedure was carried out on this animal and the antlers were processed using the same method but no microspheres were injected. These antlers were processed in the middle of the experiment to check for cross contamination during the laboratory extraction process.

In all animals, the left antler acted as the control antler and the right as the treatment antler. This was because catheterisation was conducted on the left to allow entry from the left carotid artery into the left ventricle. It was felt that this may interfere with the blood flow to the antler and that this would have less impact on the control antler than the test antler when results were interpreted.

Pilot Study

A pilot study was conducted on one stag to determine the suitability of the anaesthetic protocol, surgical procedure and to test the concept of using fluorescent microspheres.

The stag was anaesthetised as per the protocol outlined below. This anaesthetic protocol was effective and satisfactory to allow the experiment to be conducted. Catheterisation of the femoral artery and brachial artery were attempted. These were unsuccessful and the right ventricle of the heart could not be cannulated by either of these routes. The carotid artery was found to be most successful and monitoring of the pressure wave with a blood pressure transducer confirmed entry into the ventricle. Microspheres were injected at four different tourniquet tensions and the animal was euthanased and antlers removed.

Freeze sectioned antler samples were examined under a fluorescence microscope to determine microsphere presence within the antler tissue. Preliminary results using digested antler could not be produced before the remaining stags had grown their antlers to a length that required velvet-ing. It was concluded from the observation of spheres in frozen sections that there was sufficient evidence to continue with the remaining animals.

Experimental Tourniquet

The experimental tourniquet was constructed of a steel rod with a series of pegs along one edge and a spring balance (0-10kg) attached to a hook and wing nut along the other side as shown in Figure 4.1.2. The tourniquet material, attached to the hook and balance, was wrapped once around the antler (complete circle with small overlap (Figure 4.1.3)) and then secured over the appropriate peg to take up the slack with minimal tension. The tourniquet was then adjusted to the required tension by turning a wing nut attached to the bolt and hook. Friction was overcome by using lubricant and periodically rolling the device to help distribute the forces around the pedicle evenly.

Anaesthesia

The stag was restrained in an padded hydraulic crush. The left or right facial vein area was clipped and prepared for catheterisation. A 20 gauge, 48mm intravenous catheter was placed in the facial vein and held in place with cyanoacrylate adhesive. Anaesthesia was induced using propofol (Repose, propofol 10mg/mL, Norbrook New Zealand Ltd.) and ketamine (Ketamine injection, ketamine hydrochloride 100mg/mL, Parnell Laboratories New Zealand Ltd). An initial bolus of 500mg of ketamine and 150mg of propofol was mixed in a single syringe. This was followed by 400 to 600mg of propofol, sufficient to allow intubation. The stag was intubated with a size 16 ID endotracheal tube and transported to an air bed using a purpose-built sling. Anaesthesia was maintained with halothane via an out of circuit precision vapouriser (Fluotec III) driven by compressed oxygen through a small animal circle anaesthetic machine.

Anaesthetic monitoring was performed continuously and recorded every five minutes. Arterial blood pressure was monitored using a pressure transducer attached to a 22 gauge catheter in the auricular artery, normalised to the level of the left ventricle. Oxygen saturation was monitored using a pulse oximeter on the tongue. This gave variable results and was discontinued. Heart rate and character were recorded with a constant electrocardiogram with leads applied in a base-apex arrangement.

A constant infusion of dobutamine was administered to maintain blood pressure as high as possible without inducing cardiac arrhythmia. This was done because blood pressure is likely to have a significant effect on antler blood flow. Stags restrained in a crush for velvet removal have had systolic blood pressures recorded greater than 200 mmHg. Such a high blood pressure is probably induced by stress of handling. General anaesthesia usually causes a decrease in blood pressure

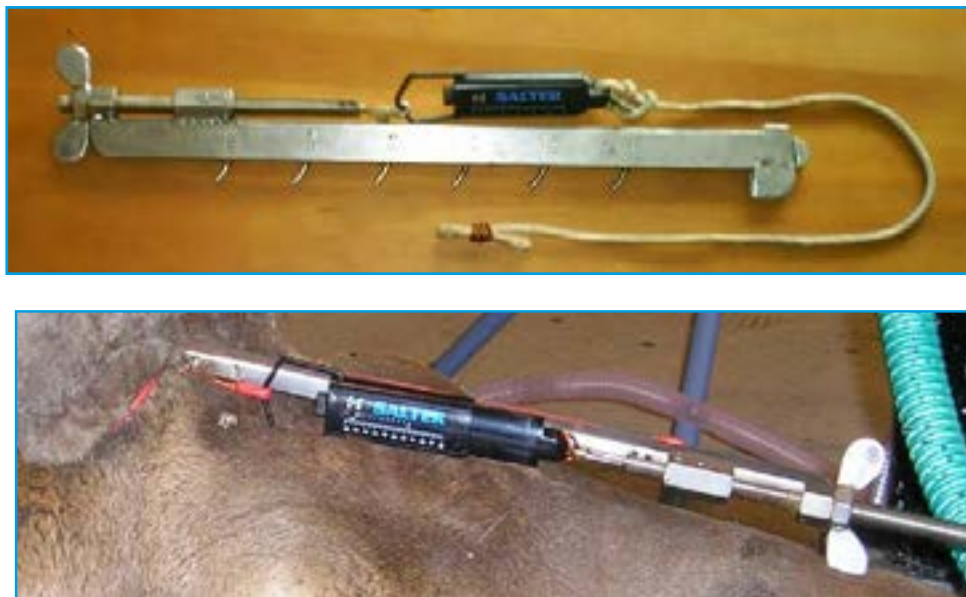


Figure 4.1.2 (above) Experimental tourniquet used to determine the effect of tourniquet tension around the antler pedicle of mature stags on the relative blood flow to the antler as determined using fluorescent microspheres, showing design and features (upper picture) and fitting onto the antler (lower picture above)



Figure 4.1.3 Fitting of the experimental tourniquet used to determine the effect of tourniquet tension around the antler pedicle of mature stags on the relative blood flow to the antler as determined using fluorescent microspheres, showing complete overlap of cord around the antler pedicle.

therefore we aimed to maintain blood pressure as close to the field situation as possible.

Cannulation of the left ventricle

The left carotid artery was palpated, dissected out and prepared for cannulation (Figure 4.1.4). A purse string suture was placed in the arterial wall and a small cut made in the centre of the purse string with small sharp scissors by repeatedly opening the ends and stretching the vessel wall. As soon as blood was seen from the cut, the cannula was forced into the opening and advanced centrally towards the heart. A blood pressure transducer was attached to the cannula and the oscilloscope trace observed during advancement as a means of determining when the left ventricle had been entered. When a ventricular trace was present, the purse string suture was drawn up and the cannula was sutured in place for injection of microspheres.

Fluorescent Microsphere injections

15µm fluorescent microspheres were used (Fluospheres Blood Flow Determination Fluorescent Colour Kit #2 Polystyrene microspheres 15µm (Molecular Probes, Invitrogen Detection technologies Eugene, Oregon, USA)) (Figure 4.1.5). Excitation and emission wavelengths for the fluorescent microspheres are shown in the table 4.1.2.

2x10⁶ microspheres were injected per treatment based on the following calculations.



Figure 4.1.4 Surgical site prepared for cannulation of the left carotid artery showing exposed artery for injection of fluorescent microspheres to determine the effect of a tourniquet applied at different tensions around the antler pedicle on the relative blood flow to the antler of mature stags.

$$N_{(min)} = 400_{(n)} / [Q_{organ}/Q_{total}]$$

Where $N_{(min)}$ = minimum number of injected FMs

(n) = number of sections that the tissue would be divided into

Q_{organ}/Q_{total} = Estimated proportional blood flow to the organ

It was estimated that the blood flow to the antlers would be at least 2% of total blood flow, and that each section would be 1% of total antler weight, therefore

$$N_{(min)} = 400(100) / 0.02 = 2 \times 10^6$$

Researchers have used greater numbers of spheres in other species. vanOosterhout (1998) used 250,000-500,000 microspheres/kg injected into the left ventricle to examine blood flow to organs in rabbits.

Using a similar dose in stags would require 6×10^7 microspheres in a 250 kg stag. The price was approximately \$150 per stag for the first equation and \$3,700 for the latter. The results from the pilot study in this project confirmed that spheres were quantifiable in the antler and using the smallest number that could be quantified was the most feasible option for the remainder of the animals.

Red microspheres were used as the control colour with no tourniquet in place in all cases. All other colours were randomly allocated to a different tourniquet tension. The person counting the microspheres was blinded to which treatment had been given for each colour in each antler except red which was always used for the control tension (no tourniquet).

Microspheres were stored in a chilly box and removed immediately before use. Each bottle was placed in a cold ultrasonic water bath for 5 minutes prior to injection. Before drawing from the bottle, the bottle was agitated for 30 seconds and the dose drawn and delivered immediately to prevent settling or clumping in the bottle or the syringe.

The pedicle of the right antler was cleaned of dirt and loose hair and smeared with approximately 20mls of obstetrical lubricant. The tourniquet was positioned around the pedicle as shown in Figure 4.1.3 and adjusted several times so as to ensure an even contact and pressure about the entire pedicle. The tourniquet was then tightened to the first randomly assigned tension.

Microspheres were injected into the ventricular cannula over one minute and time of injection and blood pressure were recorded. The injection was made slowly to allow mixing with ventricular blood (Figure 4.1.6).

The tourniquet was left in place for five minutes and then released fully for a further five minutes before tightening to the second randomly assigned tension. This procedure was repeated for six different tensions and six different fluorescent microsphere colours. The stags were then euthanised and the antlers harvested.

Microsphere recovery

Stock solutions

2.3M Ethanolic Potassium Hydroxide

3g of KOH pellets and 0.5g Tween 80 was placed in a glass beaker. Ethanol was added to the 100mL line and the solution was stirred with a magnetic stirrer until clear (20 minutes).



Figure 4.1.5 Fluorescent blood flow determination colour kit #2 showing the seven different colours in 10mL bottles as used to determine the effect of tourniquets applied at different tensions around the antler pedicle on relative blood flow to the antler in mature stags.

Table 4.1.2 Excitation and Emission wavelengths for fluorescence of different coloured Fluospheres in the Blood Flow Determination Kit as specified by the manufacturer (Molecular Probes).

	Excitation	Emission
Red	580	605
Orange	540	560
Yellow-green	505	515
Blue-green	430	465
Blue	365	415
Crimson	625	645
Scarlet	645	680



Figure 4.1.6 Injection of 2mL of yellow fluorescent microspheres into a cannula placed in the left ventricle of an anaesthetised stag to determine the effect of tourniquets at different tensions applied to the antler pedicle of mature stags on the relative blood flow to the antler.

Phosphate Buffer Solution

5.88g of KH_2PO_4 was added to 200mL of distilled water and 22.9g of K_2HPO_4 was added to 800mL of distilled water. These solutions were combined and kept as stock. For washing, 28.6mL of stock was diluted to 1000mL with distilled water.

Saturated sodium chloride solution

11.56g NaCl in 100mL water

Method of microsphere extraction

The initial plan was to follow the Fluorescent Microsphere Resource Manual recommendation for fluorimetry counting of microspheres in dissolved tissue sections.

Ten 20-gram samples were taken from various sites along the antler including the base, mid section, tynes and tip of each antler and placed in separate labelled 150mL polystyrene pots.

Samples were autolysed at room temperature for five days and then weighed accurately.

Two grams of Tween 80 was added and the samples were vortexed for 30 seconds.

Samples were placed in an oven at 50°C and vortexed daily and examined for state of digestion for three days.

After three days, there was not sufficient digestion to allow quantification of microspheres by fluorimetry. The samples remained in the oven for four weeks and were then transferred to the laboratory and stored away from light, at room temperature as no further breakdown of tissue was occurring at this time. The samples were brown and contained a large amount of particulate matter making them unsuitable for microsphere counting by fluorimetry so other methods were investigated. The possibility of direct counting of filtered spheres using a microscope was examined.

Samples were examined periodically for presence of visible microspheres and these maintained clear, spherical shape and with a sharp outline and maintained good fluorescence intensity. Each 20 gram sample was not likely to yield enough coloured spheres for statistical analysis. Therefore all ten samples from each antler were pooled for further extraction and counting. A large quantity of undissolved hair and bone tissue remained in the solution which needed to be separated from the fluorescent microspheres.

The option of proteases that could digest hair and bone without degrading the polystyrene was explored. No suitable product could be found in New Zealand and cost and potential restriction on importing from overseas along with the possibility that the method could be unsuccessful prevented this option.

Centrifugation was attempted at 2000G for 20 minutes on one sample as recommended in the

Fluorescent Microsphere Resource Manual. This required large volume centrifuge pottles and a large centrifuge. This method did not adequately separate the microspheres from the particulate matter.

The following suspension and filtration method was tested and found to be satisfactory. The equipment setup is shown in Figure 4.1.7.

A washed 1000mL cylinder was fitted with a funnel and coarse kitchen sieve.

Pooled samples were shaken for 20 seconds then poured into the sieve and funnel and forced through the sieve with a spatula to break down remaining lumps of antler. These were easily macerated using this method.

A piece of muslin cloth was placed in the sieve and funnel and the solution was again poured through and rinsed twice with phosphate buffer. The muslin and large particulate matter were discarded.

A negative pressure filtration stand was constructed as shown in Figure 4.1.7.

A 47mm diameter polytetrafluoroethylene filter with a pore diameter of 80µm (Millipore, Omnipore disc PTFE hydrophilic, France) was placed on the filter holder and the solution was run through this filter to remove particulate matter greater than 80µm. This was rinsed with phosphate buffer.

The pottles, sieves and funnel were rinsed twice with phosphate buffer solution and the washings were retained along with the samples in the cylinder and the piece of coarse filter paper.

The solution was left to settle for 30 minutes after which time there was a clear division between sediment of undissolved tissues at the bottom of the cylinder and clear solution at the top.

The supernatant was withdrawn using a pipette and run through a negative pressure filter containing A 47mm diameter polytetrafluoroethylene filter with a pore diameter of 10µm (Millipore, Omnipore disc PTFE hydrophilic, France). These were effective at trapping the 15µm microspheres suspended in the supernatant.

The sediment was then re-suspended in saturated saline solution (relative density 1.15g/cm³, calculated as ratio of weight to volume) to float remaining microspheres that were trapped in the sediment layer. The microspheres had a claim of specific density of 1.10g/cm³. This was allowed to settle for approximately 30 minutes before the supernatant was removed by suction and filtered through a new 10µm PTFE filter.

This procedure of re-suspension and filtration was repeated several times for each sample.

All equipment was washed thoroughly with hot water and detergent and rinsed with distilled water and dried between samples.

Filter papers were air-dried and placed onto a glass microscope slide then covered with a thin layer of glycerol or peanut oil and a second glass slide placed on top and taped down. Each slide was labelled with a unique ID that did not identify the treatment or antler (Figure 4.1.7, lower picture). The glass slide placed on top was etched with parallel lines to allow accurate slide scanning.

Slides were examined using a confocal fluorescence microscope under broad spectrum UV light. Slides were scanned under low power by moving the stage back and forth horizontally using the etched lines on the top slide for guidance. All the microspheres of each colour that were captured on the filter paper were counted individually using the recording sheet shown in Figure 4.1.8. An example of the view of the fluorescent microspheres is shown in Figure 4.1.9 as seen through the lens of a digital camera held onto the eyepiece of the microscope.

This method accurately differentiated six colours. Scarlet spheres could not be seen using broad spectrum UV light.

Microspheres appeared different in colour to the descriptions on the label. Individual slides with a sample of a single colour was made for each of the colours along with a slide with all the colours together. This allowed the person counting the spheres to calibrate their eye with what was observed on the sample slides compared to the test slides and to ensure that the colours could be differentiated when on the same slide.

Analysis of data

The proportion of spheres reaching the treatment antler at each tourniquet tension was calculated using Equation 1 (below). The calculation was based on the ratio of spheres reaching the treatment antler (T(A)) compared to the control antler (C(A)) when the tourniquet was placed at tension A. To account for differences in blood flow between the treatment and control antlers, this was divided by the ratio of spheres recovered when no tourniquet was applied from the treatment (T(0)) compared to the control antler (C(0)).

Equation 1:

$$\text{Proportion} = \frac{T(A)/C(A)}{T(0)/C(0)}$$

Where:

T(A) = number of colour A spheres in treatment antler at tension A

T(0) = number of red spheres in treatment antler at zero tension

C(A) = number of colour A spheres in control antler at tension A

C(0) = number of red spheres in control antler at zero tension

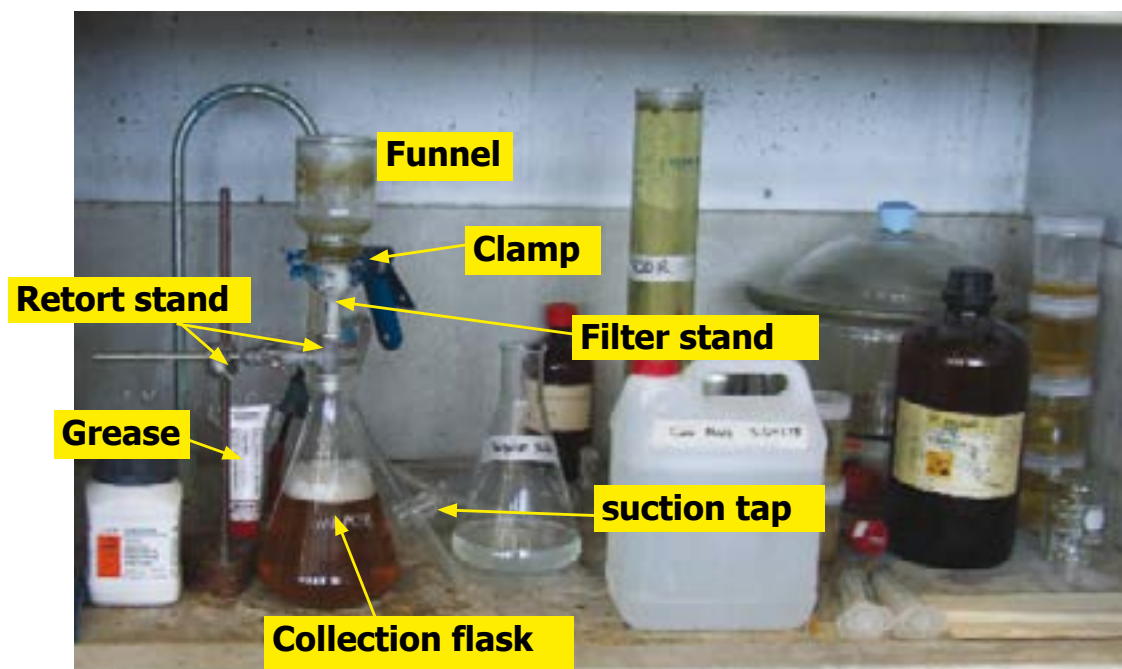
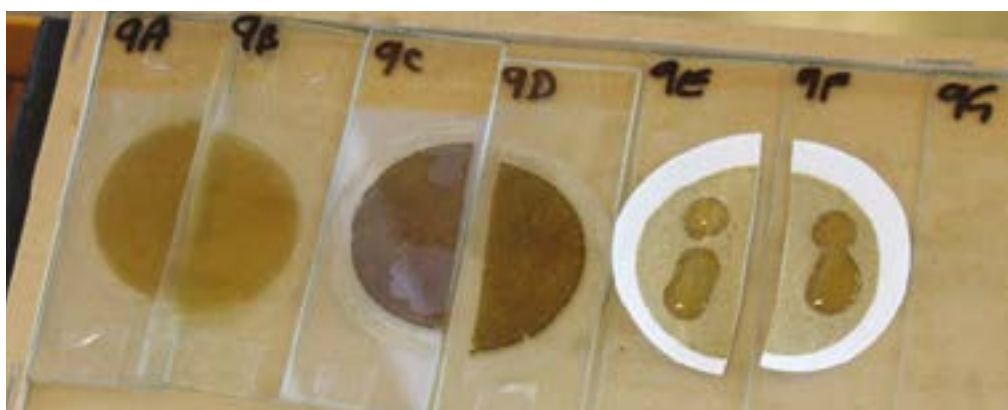
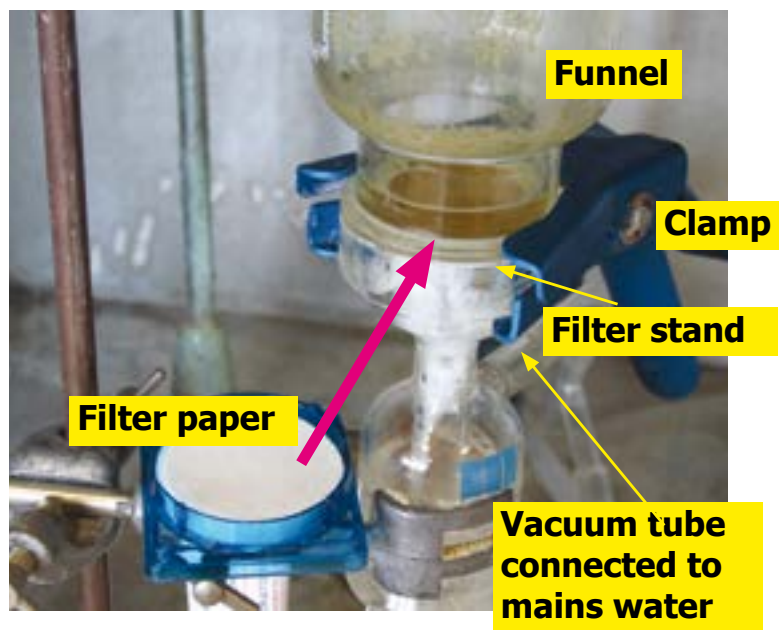


Figure 4.1.7 Set-up of negative pressure filtration system used to recover and count fluorescent microspheres from antler tissue from stags that had been injected with fluorescent microspheres while a tourniquet was applied to the antler pedicle at different tensions. Running water was used to create vacuum pressure in the collecting flask (top photograph). A 10 micron PTFE filter is placed on the stand to trap 15 micron microspheres (middle photograph). Filter papers from each filtration cut in half and mounted on glass slides for microsphere counting are shown in the lower photograph.



Results

Anaesthesia and blood pressure

Anaesthesia was successfully induced and maintained in all deer. There were no anaesthetic complications. Some deer were observed to develop cardiac arrhythmia when high doses of dobutamine were infused. These resolved by decreasing the dobutamine infusion rate.

Figure 4.1.10 shows the systolic blood pressure recorded at the time of each injection of microspheres for each deer in order of injection. In six of the nine deer blood pressure remained fairly stable between 120 and 170 mmHg. In three deer, systolic blood pressure dropped below 120mmHg and remained lowered during anaesthesia.

Quantifiable data

Four deer were able to have both left and right antlers processed and microspheres quantified using the final method. The results were consistent between these animals and the difference between control and treatment tensions was large. This number of animals proved to be sufficient for analysis of the data.

Laboratory Control

No microspheres were recovered from any of the sections analysed from the control antlers (deer 1820) showing that there was no cross contamination between samples run through the same glassware.

Microsphere tallies

Table 4.1.3 shows the total tally of microspheres and relative recovery (treatment versus control) for each tourniquet tension setting in each deer from which a full data set was acquired. Also shown in table 4.1.3 is the treatment order for each tourniquet setting and the systolic blood pressure recorded during each treatment. Deer are listed in the table in the order of experiment. Figure 4.1.11 shows the relative microsphere recovery (calculated from equation 1) for each tourniquet tension in 4 stags. Microspheres of all six detectable colours were recovered from the control (left) antler of all deer. The rate of recovery varied between deer and between microsphere colour. Red microspheres (No tourniquet) were recovered from all treatment (right) antlers. Other colours were recovered from some treatment antlers but not others.

Effect of tourniquet tension on microsphere recovery

The relative number of microspheres recovered from the treatment antler at each tourniquet tension compared to the control antler and zero tourniquet tension is shown in Figure 4.1.11. When

SLIDE	Pale red	B r i g h t red	Orange	Green	Pale blue	P u r p l e blue	Other
Total							

Figure 4.1.8 Example of the tally sheet used to quantify the number of fluorescent microspheres that were recovered following filtration of digested antler from stags that had been injected with different coloured microspheres with a tourniquet applied to the antler pedicle at different tensions

Figure 4.1.9 (right) Photograph of fluorescent microspheres recovered from antlers of stags that had been injected with microspheres of different colours with a tourniquet applied to the antler pedicle at different tensions.

View as seen under UV light using a digital camera held onto the eyepiece of the microscope at 40x magnification.

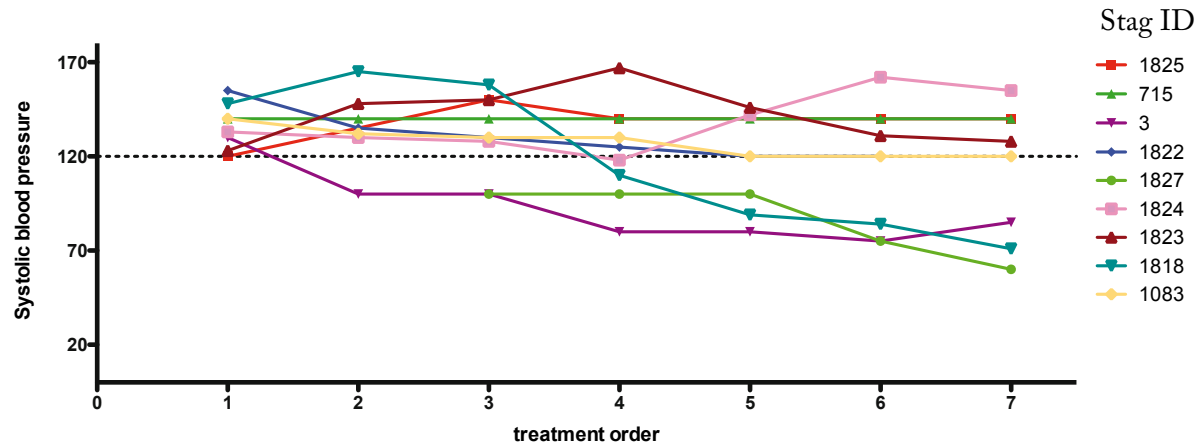


Figure 4.1.10 Systolic blood pressure recorded at the time of each injection of fluorescent microspheres in anaesthetised adult stags that had fluorescent microspheres injection while a tourniquet was applied to the antler pedicle at different tensions.

Table 4.1.3 Fluorescent microsphere counts from stags that were injected with different coloured fluorescent microspheres while a tourniquet was applied to the antler pedicle at different tensions. Table shows deer ID, tourniquet tension, order of treatment, systolic blood pressure at time of injection, absolute counts from left (control) and right (treatment) antler, and the relative proportion recovery of each colour in the treatment antler compared to control.

Deer	Tourniquet tension (kg)	Treatment Order	Colour	Blood pressure (mmHg)	Count Left antler (control)	Count right antler (tourniquet)	Proportion (Equation 1)
1825	0	1	Red	120			
Pilot	0.5	2	Crimson	140			
	1	3	Yellow	150			
	3	4	Green	140			
	6	5	Scarlet	140			
Pilot deer - Not quantified							
715	0	2	Orange	140			
	5	4	Green	140			
	6	1	Red	140			
	7	6	Crimson	140			
	8	3	Yellow	140			
	9	5	Blue	140			
Samples not quantifiable							
3	0	1	Red	130			
	3	6	Crimson	75			
	5	4	Green	80			
	7	2	Orange	100			
	8	3	Yellow	100			
	9	5	Blue	80			
Samples not quantifiable							
1822	0	1	Red	155	407	571	1
	1	3	Yellow	130	128	1	0.010
	2	5	Blue	120	159	5	0.014
	4	2	Orange	135	348	31	0.138
	6	6	Crimson	120	226	5	0.005
	8	4	Green	125	243	5	0.016
1827	0	1	Red	na	20	112	1
	1	4	Green	100	15	1	0.013
	3	3	Yellow	100	29	0	0
	5	2	Orange	na	25	2	0.014
	7	5	Blue	100	27	2	0.013
	9	6	Crimson	75	6	0	0
1823	0	1	Red	123		81	
	1	5	Blue	146		12	
	3	2	Orange	148	Not	1	
	5	4	Green	167	quantifiable	0	
	7	6	Crimson	131		0	
	9	3	Yellow	150		0	

Table 4.1.3 Continued							
Deer	Tourniquet tension (kg)	Treatment Order	Colour	Blood pressure (mmHg)	Count Left antler (control)	Count right antler (treatment)	Proportion (Equation 1)
1824	0	1	Red	133		60	
	2	3	Yellow	128		0	
	4	5	Blue	142	Not quantifiable	2	
	6	2	Orange	130		1	
	7	6	Crimson	162		7	
	8	4	Green	118		0	
1818	0	1	Red	148	45	74	1
	2	5	Blue	89	186	5	0.016
	4	3	Yellow	158	129	4	0.018
	6	6	Crimson	84	96	1	0.006
	8	2	Orange	168	70	2	0.017
	9	4	Green	110	68	1	0.009
1083	0	1	Red	140	39	138	1
	1	4	Green	130	17	0	0
	3	5	Blue	120	48	4	0.024
	5	3	Yellow	130	13	0	0
	7	6	Crimson	120	47	0	0
	9	2	Orange	132	43	2	0.013
1820 (Control)	Nil		None injected	NA	0 All samples zero	0	

Table 4.1.4 Relative fluorescent microsphere recovery (%) from antlers from stags that had been injected with different colours of fluorescent microspheres while a tourniquet was applied to the antler pedicle at different tensions. The number is compared to the control antler and zero tourniquet tension.

Tension (kg)	Deer				
	1 (1822)	2 (1818)	3 (1827)	4 (1083)	5 (1820)
0	100	100	100	100	0
1	1.0		1.3	0	
2	1.4	1.6			
3			0	2.4	
4	13.9	1.9			
5			1.4	0	
6	0.6	0.6			
7			1.3	0	
8	1.6	1.7			
9		0.9	0	1.3	

a tourniquet was applied at a tension of 1 kg or greater, the number of microspheres that were recovered from the treatment antler was significantly reduced to $1.6\% \pm 3.0\%$ (standard deviation) (range 0 to 13.9%) when compared to the control colour and control antler.

All except one of these values was 2.4% of control (no tourniquet) value or less. One sample from animal 1822 was an outlier and had a high level of leakage of 13.9% when the tourniquet was placed at a tension of 4 kilograms.

Within the parameters used in this study there was no significant linear regression fit for the data of the standard curves available using Prism 4 software.

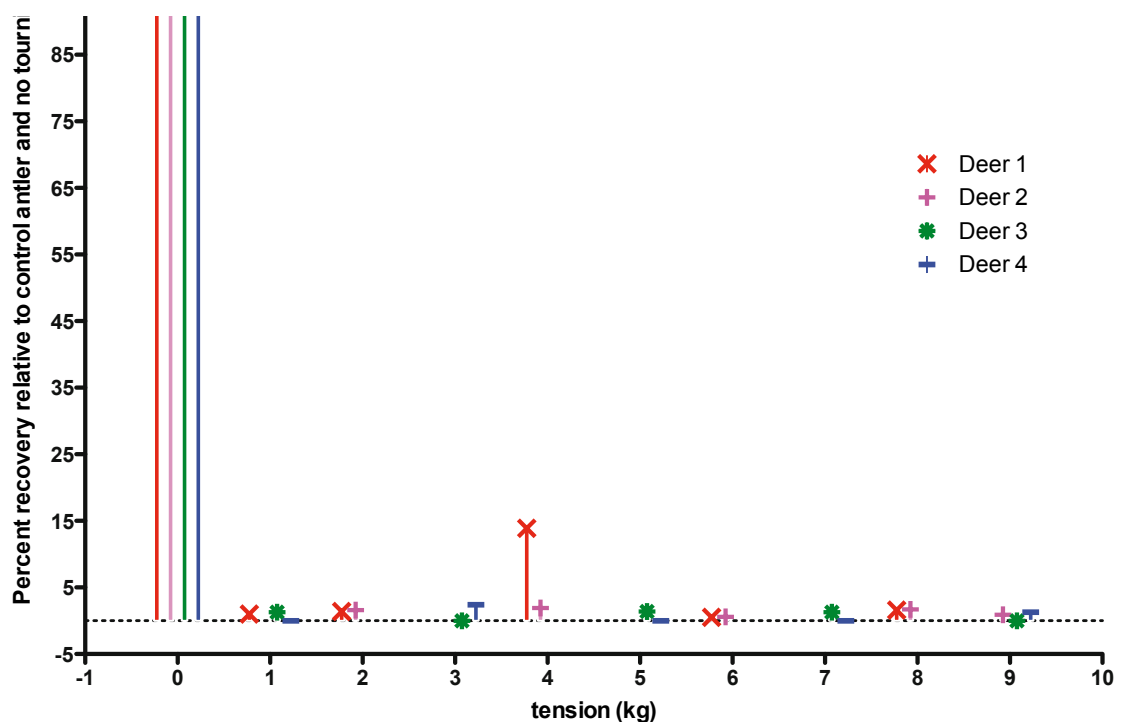


Figure 4.1.11 Relative number of fluorescent microspheres recovered from antlers that had a tourniquet applied to the antler pedicle at different tensions during injection of different coloured fluorescent microspheres. The relative recovery rate is the number of microspheres recovered from the treatment antler compared to the control antler and compared to the treatment antler when no tourniquet was applied (zero tension).

Four deer had data recovered but not all deer had the tourniquet applied at each tension.

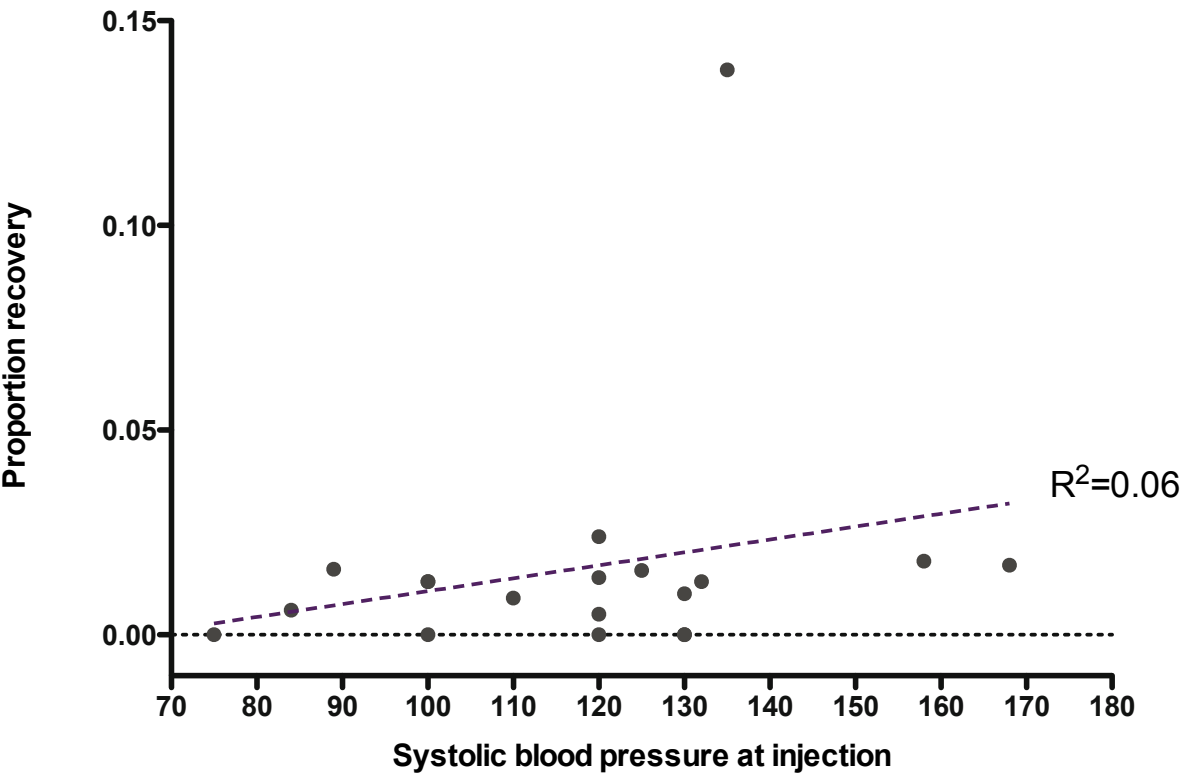


Figure 4.1.12 Relative number of fluorescent microspheres recovered from antlers that had a tourniquet applied to the antler pedicle at different tensions during injection of different coloured fluorescent microspheres compared to systolic blood pressure recorded at the time of injection. Each point represents an antler.

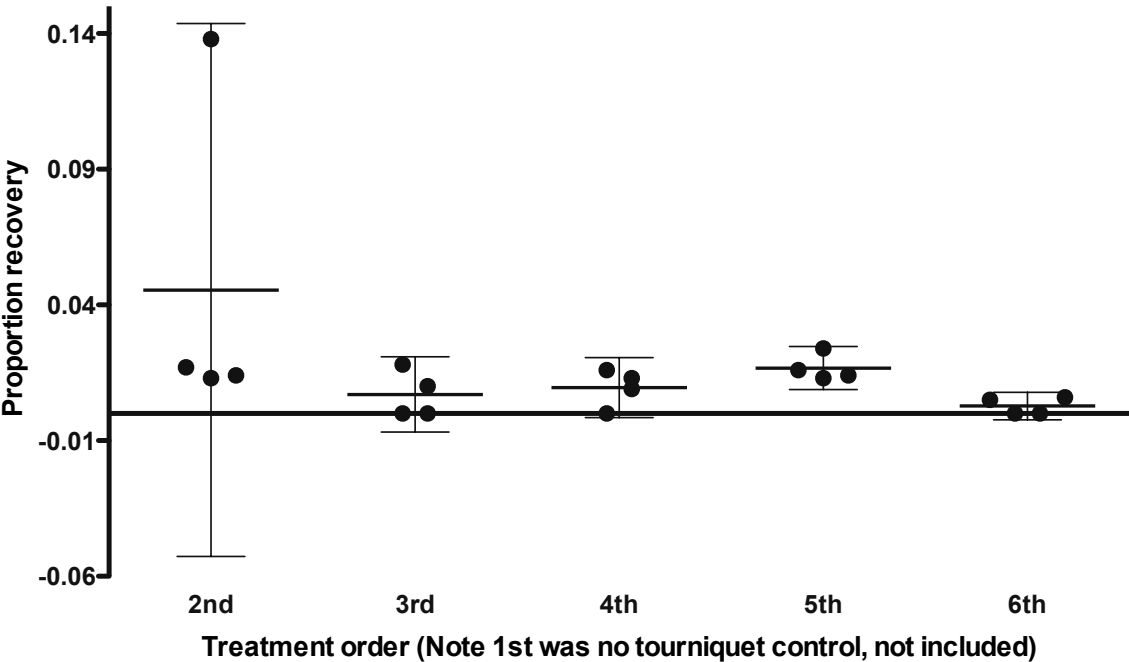


Figure 4.1.13 Relative (treatment/control) recovery rate of fluorescent microspheres from deer antlers for each consecutive injection event following intracardiac injection of 6 different colours of 15µm polystyrene fluorescent microspheres. The first injection was the control (no tourniquet) and results are not shown here, subsequent injections were made after a tourniquet was placed on the antler pedicle.

Effect of blood pressure on microsphere recovery rate

The relationship between blood pressure and microsphere recovery was tested (Figure 4.1.12). The straight line regression correlation between systolic blood pressure and proportion recovery was not significantly different from zero ($R^2 = 0.06$, $p = 0.3132$).

Effect of treatment order

Treatment order was ranked from first to sixth. Relative microsphere recovery for each treatment in order is shown in Figure 4.1.13. One-way ANOVA found no significant effect of treatment order and no significant differences between any of the orders of treatment. An outlier value of 13.9% was calculated from the first treatment applied to deer 1822.

Discussion

Effect of tourniquet tension on antler blood flow

The results from this study show that applying a tourniquet around the antler pedicle, at any tension at or above 1kg of tensile force, significantly reduced blood flow to the intact antler of anaesthetised mature red deer stags.

In all deer, on all but one occasion, the number of microspheres reaching the antler when a tourniquet was in place was reduced by at least 97.5% (Equation 1). This result supports the theory that the arterial supply to the antler is located peripherally at the level of the pedicle and that a tourniquet applied around the pedicle can occlude the majority of arterial blood flow to the antler.

On one occasion, when the tourniquet was applied at a tension of 4kg, 13.9% of the injected microspheres entered the antler. On examining the order of treatments, this was the first treatment in the first animal. It is possible that on that occasion, the tourniquet was not placed adequately as the researchers were still becoming familiar with the equipment. On all subsequent treatments, including later treatments on the same deer, the proportion of microspheres entering the antler was 2.4% or less. Regardless of the reason for the outlier, it represents a situation that could occur in the field. If the leakage was due to inaccurate placement or slippage after placement, it is feasible that this could occur during velveting using current tourniquet methods. Leakage between the time of lignocaine placement and antler harvest may account for noncompliant residue levels in some antlers.

At tensions greater than one kilogram, there was no correlation between tourniquet tension and microsphere recovery from antlers. Even at tensions of 9 kilograms, some microspheres reached the antler and this was not significantly different from the number that reached the antler at one kilogram. It appears that there is maximum effect that can be achieved by using a tourniquet and that this level is reached at low tourniquet tensions.

It is possible that a relationship between tourniquet tension and microsphere recovery may have been seen if tensions less than one kilogram were used. The device in our system was too heavy to allow settings below one kilogram. It was not known what tensions would be required before the commencement of the study so we aimed for a tension to include the range expected in general practice.

The leakage of microspheres at all tensions up to 9 kg suggests that either the tourniquet can not fully occlude all the blood vessels as they traverse the pedicle or that the blood reaches the antler by an alternative route. On the pedicle surface, arteries may be located within anatomical structures that protect them from the direct pressure of the tourniquet. Another possible explanation is that a collateral blood supply may exist that is not occluded by the tourniquet. If this was the case, the proportion of blood entering the antler by an alternative route would be less than 2%. Because the microspheres were injected into the left ventricle of the heart, it would allow them to travel into the antler via all available routes. This possibility could be further investigated using arterial casts.

Effect of arterial blood pressure

There was no effect of systolic blood pressure on the proportion of microspheres that reached the antler. There were insufficient data to perform a chi-square test, main effects test or multivariate regression analysis to separate the confounding effects of blood pressure, treatment order and tourniquet tension. Basic analysis of the relationship between blood pressure and recovery rate from all treatment antlers showed no significant difference from a zero relationship. Blood pressure would be expected to affect the tension required to prevent arterial flow to the antler. The highest systolic blood pressure recorded during these experiments was 168 mmHg. Values around 120 to 130 mmHg were more common and in one deer systolic blood pressure was consistently 100 mmHg or less (Figure 4.1.10). These pressures are considerably lower than systolic blood pressures that have been recorded from stags restrained in a crush (Woodbury et al, 2005). It is possible that the stress of restraint would raise the stag's blood pressure to a level where the tourniquet no longer prevents blood flow when it is applied at low tensions of one to two kilograms. In this experiment, in a deer that had a blood pressure of 168 mmHg, a tourniquet at a tension of 2 kg was sufficient to reduce microsphere recovery to 1.7% of the control antler. This suggests that relatively low tensions would prevent blood flow even if blood pressure was 200 mmHg as recorded by Woodbury et al (2005).

While attempts were made to keep systolic blood pressure as high as possible using dobutamine, this was not always achieved. Prolonged administration of dobutamine resulted in serious cardiac arrhythmia in a number of stags requiring the infusion to be discontinued.

Effect of treatment order

There was no significant treatment order effect on the number of microspheres in the antler. This was tested because over time, the tourniquet could have compressed the blood vessels and tissues

around the pedicle so that a lower tension would be required to occlude the arterial supply to the antler. Observation of the antler stump after cutting the velvet and removing the tourniquet often reveals that pulsatile arterial haemorrhage takes seconds to minutes to commence. It is not known whether occluding the vessels, releasing and re-occluding affects the blood flow in the intact vessels between tourniquet applications or what the effect would be during subsequent compressions. In order to test this it would be necessary to have no-microsphere controls randomly ordered between treatment controls and comparing the relative proportion (treatment antler vs control antler) with the initial control measurements before any compressions were applied. In the field, the tourniquet would usually only be applied once.

There was confounding between treatment order and blood pressure as blood pressure tended to decline with increasing treatment order in all deer. These factors combined would be expected to cause a decrease in the number of spheres reaching the antler or to decrease the tension required to prevent blood flow to the antler. This was not the case in the current study which suggests that systolic blood pressure and treatment order do not affect antler blood flow within the limits of the tensions that we were applying. An effect may have been seen at lower tensions.

Effect of general anaesthesia

General anaesthesia may also alter the dynamics of the blood flow to the antler. Propofol and ketamine both have a short duration of action and the effects of these induction drugs would be minimal by the time the microspheres were injected. Halothane causes cardiovascular depression but the effects of peripheral blood flow are not known in deer. In humans, vasodilation is most common, resulting in decreased blood pressure and bradycardia. This could have a potentially unpredictable effect as blood vessels may be dilated allowing a greater volume of blood flow to the periphery but decreased blood pressure would mean a lower tourniquet tension would be required to stop the flow. The effects of anaesthetics on arterial smooth muscle at the level of the pedicle are not known.

Use of fluorescent microspheres

Fluorescent microspheres can be used to quantify antler blood flow and microspheres 15 μm in diameter will be trapped in the capillary bed of the antler. The digestion, extraction and recovery procedures recommended by the Fluorescent Microsphere Resource Centre requires modification for application to velvet antler.

We aimed to determine the relative number of spheres along the length of the antler, and thus improve our understanding of the anatomy of the microvasculature of the antler, by taking samples from ten different sites. Low recovery rates necessitated the pooling of these samples so that only whole antler analysis was possible.

A low concentration of microspheres was injected due to the cost of the microspheres. Because intracardiac injection was used, the distribution of the spheres was throughout the body thus requiring enough circulating microspheres to reach all the peripheral tissues. The proportion of systemic

blood going to the antlers was not known before the commencement of the study and an estimate of 2% was used. Better recoveries may have been achieved if a larger number of microspheres was used for each injection or if injections were made into the carotid artery. The latter option would not have allowed for equal flow to both the treatment and control antlers for comparisons. Improving the method used to recover and count the microspheres may also result in better recovery rates at the same injected volume. The recovery rate and accuracy for the extraction method was not calculated. A blank antler could have been spiked with known volumes of microspheres and extracted using the same method as the treatment samples to determine recovery rates. Repeated spiking with the same volume would allow for determination of the repeatability of the test. The absolute recovery rate was not critical to the calculations for the purposes of this study as absolute volume of antler blood flow was not examined and having both a within antler control and a between antler control allowed for correction for different recovery rates between antlers.

There were several difficulties encountered during recovery and quantification of the microspheres. The tissues took considerably longer to break down than anticipated. The recommendations in the fluorescent microspheres resource manual suggested that the tissues should break down within 48 hours in ethanolic potassium hydroxide, whereas the antler was not fully broken down even after several weeks of digestion. This may have been due to the calcified nature of the antler and the presence of hair and hair follicles. Examination of slides of the undigested portion revealed intact hair follicles. A stronger concentration of potassium hydroxide, for example 4 molar rather than 2.3 molar would be recommended for future experiments. Aqueous potassium hydroxide may also be more effective than ethanolic potassium hydroxide.

Polystyrene microspheres are prone to settling and clumping together so efforts were made to avoid clumping and to keep samples consistent between animals. Methods to prevent clumping included vibrating the sample in an ultrasonic water bath for five minutes, agitating the bottle vigorously before injection, minimising time in the syringe and injecting slowly over fifteen seconds to allow mixing in the left ventricle. It appears that this was not always effective as demonstrated by the variability in the results in the control antler. As the same volume of microspheres was injected each time, the control antler should have had similar numbers for all colours but this was not the case as shown in Table 4.1.3. Deer 1822 had 407 red microspheres in the control antler and only 128 yellow microspheres, whereas deer 1818 had a similar number of yellow microspheres (129) but 45 red microspheres in the control antler. These differences were accounted for in Equation 1 as it was assumed that the proportional distribution of blood flow to the treatment antler compared to the control would be similar for each injection. Having a within-antler and between-antler control allowed for variation in the injected concentration of microspheres. This variation does highlight the point that the number of injected microspheres cannot be accurately predicted so caution should be exercised if calculation of absolute blood flow is to be performed.

Due to the amount of trial and error that was undertaken with tissue extraction, several samples were not able to be used in the final methodology. A flood that occurred in the laboratory also ruined a number of prepared slides before they had been examined under the microscope rendering them unable to be analysed. These factors resulted in complete data sets from four test animals and one blank control animal. While this number of animals was sufficient to give an indication of the effect of a tourniquet, having a larger number of usable samples may have identified other

outlier values or trends between microsphere recovery rate and tourniquet tension, blood pressure or treatment order. There was little ability to perform valid statistical comparisons.

During the pilot study, various routes and types of cannulae were investigated to determine the most appropriate site and method of cannulation in order to inject into the left ventricle of the heart. The Right femoral artery was prepared. However, this proved too small and fragile for the procedure. The right brachial artery was examined, but was too deep relative to muscle masses and small in size. The left carotid artery was relatively accessible and straight forward to isolate and cannulate.

Conclusions

Applying a tourniquet at a tension of one kilogram or greater prevented at least 97.5% of microspheres from entering the antler after injection into the left ventricle in all except one case.

The tourniquet was not completely reliable and one case when the tourniquet was recorded as being applied at 4kg tension, 13.4% of the microspheres entered the treatment antler. This may have resulted from poor tourniquet placement or slippage on that occasion and may reflect a situation that could occur in the field.

At tensions greater than 1 kilogram, there was no correlation between tourniquet tension and microsphere recovery rate.

4.2 Angiography/cinefluoroscopy to explore antler circulation and determine the effect of tourniquet tension on antler blood flow in adult deer

Introduction

Contrast radiography of the arterial supply to the antlers has been performed previously (Suttie et al, 1985). In that experiment, serial still X-ray angiograms were taken after injection of contrast medium into the exteriorised carotid artery in young red deer. The results showed that the main arterial supply to the pedicle comes from the superficial temporal artery and also demonstrated that angiograms could be generated using contrast iodine injected into the carotid artery. Continuous fluoroscopy (real-time continuous low intensity x-ray) and video capture is commonly used in veterinary and human medicine and research but has not been described previously for deer antlers.

The vascular pattern distal to the pedicle has not been described in adult red deer and while it is believed that the major blood supply comes from the superficial temporal artery, branching and patterns of anastomoses beyond the level of the pedicle are not well understood. This study aimed to use continuous fluoroscopy of a contrast angiogram to determine the effects of different tourniquet tensions on the blood flow to the antlers of deer. It also aimed to create images of the vascular system across the pedicle and within the antler of mature red deer.

Materials and method

Two adult stags were used for this study on separate days. One stag had the right antler imaged and the other stag had both antlers imaged.

On each occasion, anaesthesia was induced with intravenous ketamine and propofol, using the same protocol as that described in Chapter 4.1, and the deer was intubated and carried on a sling into a horse float for transport to the Massey University Veterinary Teaching Hospital sheep surgery. Anaesthesia was maintained with intravenous boluses of propofol and ketamine during transport. On arrival at the surgery the stag was connected to a rebreathing circle anaesthetic machine and anaesthesia was maintained with halothane in oxygen. A catheter was placed in the auricular artery and a pressure transducer attached for measurement of arterial blood pressure. This was normalised to the level of the left ventricle. An electrocardiogram was recorded.

The carotid artery was catheterised as described in Chapter 4.1. Initially a catheter was guided into the left ventricle as for Experiment 4.1. No contrast was seen when the antler was imaged while the contrast was injected at that site (Table 4.2.1 recordings a3,a4,a5,a6). The foot was also imaged to see whether contrast medium could be detected at this site and none was seen. For all



Figure 4.2.1 Mature stag positioned for fluoroscopic examination of the velvet antler after injection of contrast iodine without or with a tourniquet applied to the antler pedicle. Anaesthetised stag in the Massey University teaching hospital with the Philips BV Libra digital C arm fluoroscope receiving plate positioned against the antler



Figure 4.2.2 Pania Flint and Radiographer Angela Hartman observe the real time fluoroscopy videos with an anaesthetised stag on the table.

remaining recordings, the carotid artery on the same side as the study antler was catheterised with the catheter directed rostrally towards the antler. This proved a satisfactory site for injection of contrast.

Fluoroscopy (real-time continuous x-ray) was performed using a Philips BV Libra digital C arm fluoroscope (Figures 4.2.1 and 4.2.2). 50mL of Iopromide 76.9% (Ultravist-370 equivalent to 370 mg iodine/ml, Schering, AG Germany) was injected by rapid bolus while recording perfusion of the ipsilateral antler with fluoroscopic examination. The same experimental tourniquet that was used in the microsphere study (Chapter 4.1) was applied to the study antler and the tension varied between each recording.

The tourniquet device had to be held away from the pedicle itself as it obscured the view of the angiogram. This resulted in unequal contact around the pedicle. For this reason, and also to determine whether positioning of the tourniquet made a difference to the amount of blood flowing into the antler, different locations for the tourniquet device were used, caudal, medial and rostral.

Real-time moving images were observed on a screen during fluoroscopy (Fig 4.2.2). These were recorded onto in-built memory (recordings a3-a6) or onto CD (all other recordings). Videos were analysed at a later date. At the completion of the recordings, stags were euthanased with Pentobarbitone while still under general anaesthesia.

Results

Results and details of experiment recordings are shown in Table 4.2.1.

Injection of contrast iodine into the left ventricle of the heart did not produce an image in the antler. Injection of contrast iodine into the carotid artery produced a clearly visible image in the ipsilateral antler as shown in Figure 4.2.3

No tourniquet

Four recordings were made with no tourniquet. In deer 1 a lateral and a cranio-caudal view were taken on the right antler only. In deer 2, a lateral view was taken of the right antler and of the left antler.

When no tourniquet was applied, Contrast iodine could be seen as black contrast in the arterial system of the antler within a few seconds of injection (Table 4.2.1, recordings b1, b2, c3 and c9). The arterial outline lasted 5 seconds and was followed by a period of 2 seconds when no contrast was visible. A second vascular pattern believed to be the venous drainage then became visible. This second flush remained visible for 10 seconds. After this time no contrast could be seen in the antler.

Effect of tourniquet

Deer 1

Tourniquet tension 1.25 kilograms

When a tourniquet was applied at a tension of 1.25 kg at the caudal aspect of the pedicle, contrast could be seen in the antler after injection (recording b3). The contrast was less intense than that seen when no tourniquet was used and persisted for longer (22 seconds, compared with 5 seconds with no tourniquet). Venous filling was also seen and this started to appear at the same time as the arterial image disappeared. A venous pattern remained visible for the remainder of the recording time (5 minutes).

When the tourniquet was applied on the medial aspect a similar pattern was seen (recording b7). Contrast could still be seen in the arterial system for approximately 5 seconds. The arterial outline disappeared more quickly than for caudal placement. Contrast remained visible in the venous system for the duration of the recording (30 minutes).

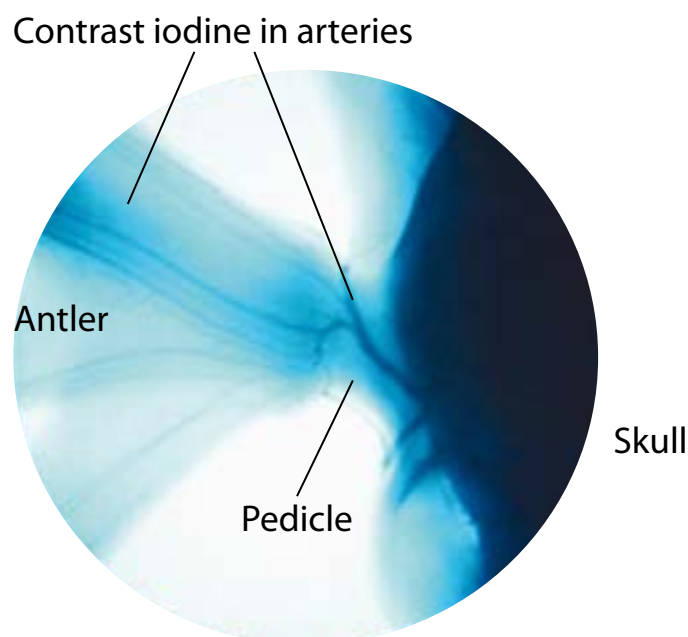


Figure 4.2.3 Still image captured from fluoroscopic study of the velvet antler of a mature stag after injection of contrast iodine into the carotid artery on the same side. Picture is of the arterial pattern at the level of the pedicle and lower antler.

Table 4.2.1 Deer angiography results showing record number, radiographic view, blood pressure and time during recording, site of injection, observations during injection and notes recorded.

Animal 1819; Right antler; Pedicle 195mm

Record	Ref	View	Tourn.	BP	Vol	Site	Time	Results
			(kg)		(mL)			
a3	Clinic	Lateral	None		45	LV	11:45	no contrast seen
a4	Clinic	Lat	None		50	LV	12:10	no contrast seen
a5	Clinic	Foot	None		50	LV	13:15	no contrast seen
a6	Clinic	Foot	None		50	LV		no contrast seen
b1	AVEQ02	Lat	None		50	L carotid		Time: 9s, visible contrast; 14s, contrast disappeared; 16s, reappear (venous); 26s, all gone
b2	AVEQ03	Cranial	None		50	L carotid		Time: 10s, visible contrast; 15s, gone; 17s, appears again, 27s, gone but not as clear as lateral view.
b3	AVEQ04	Lat	1.25	130	50	L carotid	13:35	Time: 08s contrast appears caudally (arteries thinner than record B2; 12s spread to cranial region. 30s arterial gone, venous appearing. Venous faint persisted for more than 5 minutes
b4	AVEQ06	Lat	2.0	133	50	L carotid	13:45	Time: 08s, contrast seen caudally arterial; 12s, fine contrast arterial cranially; 18s, none seen, 22s, venous visible, 49s, venous still visible.
b6	AVEQ07	Lat	2.0	139	50	L carotid	14:00	Time: 15s contrast seen below tourniquet, not seen in antler at any time during recording.
b7	AVEQ08	Lat	1.25	122	35	L carotid	14:07	Time: 13s, contrast visible then disappeared, 15s contrast visible again; 20s, disappeared; 30s, venous appearing. Venous faint but remained present for more than 20 minutes.
b8		Lat	2	156	50	L carotid	14:30	No contrast seen in antler at any time.

Animal1052; Left antler; Pedicle 140mm

Run		View	Tourn.	BP	Vol	Site	Time	
c2		Lat	None	128	50	L carotid	11:40	good image, contrast in antler
c3	Deer	Lat	1.1	122	50	L carotid	11:49	No contrast seen, video N/A*
c5		Lat	0.5	122	50	L carotid	12:00	No Contrast seen. video N/A
c6	Angio	Lat	0.1	126	50	L carotid	12:05	Contrast seen, video N/A
c7	Day 2	Lat	0.1	120	50	L carotid	12:10	Contrast seen, video N/A
c8		Lat	1	110	50	L carotid	12:20	No contrast seen, N/A

Animal 1052; Right antler; Pedicle 145mm

Run		View	Tourn.	BP	Vol	Site	Time	
c9	Deer	Lat	None	Low	50	L carotid	13:20	Contrast seen, video N/A
	Angio			batt				
c10	Day 2	Lat	0.1		50	L carotid	13:25	Contrast seen, video N/A
c11		Lat	0.5		50	L carotid		No contrast seen, videoN/A

BP: Systolic blood pressure (auricular artery normalised to level of left ventricle)

Lat: lateral view

LV: Injection made into the left ventricle of the heart

L carotid: Injection made into the left carotid artery

Foot: Radiographs were taken of the foot in attempt to visualise circulating contrast after left ventricular injection

Times are from start of film, not time of injection.

*Formatting problems prevented recordings

Deer One

Tourniquet tension at 2 kilograms

When the tourniquet was applied at a tension of 2 kg with the device at the caudal aspect of the pedicle (recording b4), contrast could be seen at the caudal aspect of the lower antler and spread to the cranial aspect over 4–5 seconds. Contrast was visible arterially in the antler for 10 seconds and then disappeared. Venous filling was seen 4 seconds later and persisted for the duration of recording (49 seconds).

When the tourniquet was applied medially on two separate recordings (b6 and b8) no contrast was seen in the antler at any time. Contrast could be seen filling the vessels below the level of the tourniquet and on the caudal area of the head.

Deer Two

Videos were not available for full analysis due to formatting problems so the tabled results are from records of contrast presence or absence that were taken at the time of the experiment.

In both antlers, contrast was seen in the antler when no tourniquet was applied. In the left antler no contrast was seen when the tourniquet was applied at 1.0 (recordings c3 and c8) or 0.5 kg (recording c5). Contrast was visible at 0.1 kg (recordings c6 and c7).

In the right antler, only three recordings were made. Contrast could be seen in the antler at 0.1 kg but not at 0.5 kg.

Discussion

Fluoroscopy is useful for studying the effects of tourniquet tension on antler blood flow for a number of reasons. Firstly, few animals are required because multiple treatments can be applied to a single animal. This is possible because of the rapid elimination of the contrast material from the vasculature (approximately one circulation time). The antler does not need to be removed as for fluorescent microsphere or lignocaine quantification and results are seen instantly compared to other methods that take up to 2 weeks for results to be reported. This allows for alteration of the treatment in response to previous results within the same experiment. The deer is fully anaesthetised meaning that this relatively invasive procedure is performed in an unconscious animal eliminating any pain or stress that might be inflicted. Finally, capture of images in digital video format allows for later detailed analysis.

The major disadvantage of this method is the relative lack of sensitivity. A large volume of concentrated contrast must be injected as a rapid bolus as it can only be visualised in the antler at high vascular concentrations. If only a small amount is able to pass under the tourniquet into the antler this is likely to go undetected. This is likely to be significant in terms of lignocaine residue in velvet.

The need for a high dose as a bolus was evidenced when attempts were made to inject the contrast into the left ventricle. No image was seen in the antler or the foot when this site was used. Images of sufficient quality were only achievable when the injection was made into the cervical carotid artery supplying the antler under study. The left ventricle was initially chosen as the injection site so that this experiment could be better correlated with the fluorescent microsphere study. In addition, it is not known whether the entire arterial supply to the antler is via the carotid artery or whether another supply also exists. Injecting into the left ventricle would allow for all of the possibilities.

The use of digital subtraction angiography (DSA) would probably have improved the visualisation of the vessels and comparison between tourniquet tensions when partial flow was detected. Digital subtraction is usually performed on multiple still frames. As the time frame for filling of the vessels was not known in advance, the use of constant fluoroscopic recording gave assurance that the entire filling and emptying periods would be captured. It may be possible to perform digital subtraction using the data already captured in this study but the quality is likely to be poor.

The results from Deer 1 demonstrate the importance of positioning of the tourniquet device. Initially the tourniquet was applied at the caudal aspect of the pedicle to avoid obscuring the pedicle. This resulted in a gap between the tourniquet device and the pedicle. Attempts were made to overcome this by overlapping the tourniquet rope but this was likely to have been insufficient to ensure proper contact around the entire pedicle. It is likely that this was the reason that contrast was visible in the antler at 1.25 and 2 kg when the tourniquet was placed in this position. When the tourniquet was moved to the medial aspect as for the fluorescent microsphere study, contrast could still be seen at 1.25 kg but not at 2 kg tourniquet tension. This incomplete compression around the pedicle due to inappropriate placement may explain why a high number of fluorescent microspheres were seen in one sample when the tourniquet was placed at 4 kg (Chapter 4.1).

The images of the arterial system show that the larger vessels were located at the caudo-lateral aspect of the pedicle. Applying a lower tourniquet tension allowed flow in these vessels but prevented flow at the cranial aspect of the antler. This suggests that the caudo-lateral region is the most important area to ensure adequate tourniquet contact and pressure.

A tourniquet applied with inadequate tension or with uneven distribution of tension caused contrast to be retained in the vasculature for a prolonged time. When the tourniquet was applied at 1.25 kg, contrast could still be seen in the antler, albeit faintly, for 30 minutes after the start of recording. Retention of contrast was also seen when the tourniquet clamp was placed caudally at 2 kg. This finding suggests that arterial blood entering the antler that has lignocaine residue may be retained for a longer period of time than if no tourniquet was applied at all. This could lead to the accumulation of lignocaine in the antler over time as presumably arterial blood is able to enter the antler but venous drainage is impaired. Hydrostatic back-pressure is likely to limit the amount of new arterial blood entering the antler and this is supported by the slower arterial filling, prolonged arterial time and delayed venous filling when the tourniquet was applied at 1.25 kg.

This finding of preferential occlusion of the venous flow compared to the arterial flow was unex-

pected as it was believed that the arterial supply is peripheral and the venous drainage is central through the antler. One possible explanation could be that venous drainage from the antler is redirected into peripherally placed veins before crossing the pedicle and that drainage at the level of the tourniquet is in fact around the outside of the bony pedicle and not through the centre. Distinct, large vessels running along the length of the antler itself were seen filling with contrast two seconds after arterial filling had disappeared (Figs 21-22). This was assumed to be venous filling after contrast had passed through capillary beds in the antler. These larger vessels show that venous drainage is not entirely through a central sinus structure as previously thought.

Conclusion

Applying a tourniquet at a tension of 2 kg prevented visible iodine contrast from entering the antler provided the tourniquet was placed against the medial aspect of the antler.

Tourniquets placed at lower tensions or where a gap occurs at the caudal aspect of the antler result in slow filling and prolonged retention of a smaller amount of contrast in the antler

4.3 Observations of the effects of a tourniquet on haemorrhage during velvet antler removal in adult stags

Introduction

The experiments in Chapters 4.1 and 4.2 have both used anaesthetised stags and intravascular agents, fluorescent microspheres and iodine contrast respectively, to determine the effects of a tourniquet on blood flow to the intact antler. This study aimed to directly observe the degree of haemorrhage that occurs immediately after the velvet antler is removed from awake, standing stags.

When the stag is under general anaesthesia (and lying down), the volume and pressure of the blood supplying the antler may be altered from that of the standing stag. Approximately half of all stags velveting in New Zealand are sedated for velvet removal and the remainder are velveting under physical restraint in a crush. Woodbury et al (2005) measured systolic blood pressures up to 200 mmHg in the physically restrained stag. High blood pressure and a tendency to move around may alter the effect of the tourniquet in the awake animal. The position of the head relative to the body is also different in the physically restrained deer, being the highest point and raised above the level of the heart to a greater degree than during recumbency. This would result in blood pressure at the level of the pedicle being less than systolic pressure measured at the level of the heart. Venous pressure in the antlers would also be lower in the standing stag compared to one that is recumbent.

This study aimed to clinically assess the degree of haemorrhage that occurs immediately after the velvet antler is removed in physically restrained stags.

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Materials and methods

Two mature sire stags were used for this experiment. The observations were made at the Massey University Deer Research unit on 1 November 2005 when the stags were at the normal stage of antler growth for velvet harvest.

The stag was restrained in a hydraulic crush specially designed for handling deer. One antler at a time was subjected to the following procedure.

1. The tourniquet device that was used in Chapters 4.1 and 4.2 was applied and tightened to 10kg force (five times the minimal tension from 4.2, which should stop all subcutaneous blood flow) with the device placed medially and protruding backwards.
2. Lignocaine 2% was injected at a dose of approximately 1mL/cm pedicle circumference in a complete ring of overlapping blebs around the base of the pedicle.

3. A wait time of four minutes was allowed.
4. Antler was removed using a meat saw.
5. The tourniquet tension was gradually reduced and then the tourniquet was removed while filming. The cut surface was observed for arterial blood flow. Arterial blood was recognised by colour and increased pressure (a fountain that was raised above the height of the cut surface).
6. The tourniquet was released fully and then reapplied and gradually tightened until arterial bleeding ceased
7. A slice was cut from the proximal end of one antler from each stag, as described by the NZFSA, and sent to AsureQuality Limited, Wellington for testing to GLP standard for residue testing.

Results

Details of the experiment and the results are shown in Table 4.3. In both cases, the tourniquet had to be completely released before any arterial haemorrhage was seen from the cut surface of the antler. When the tourniquet was released, this bleeding was evident with large, pulsation arterial spurting seen from the periphery of the cut antler.

When the tourniquet was re-tightened, tensions of less than 2kg were sufficient to halt the arterial spurting.

Table 4.3 Details of velvet removal and observations of haemorrhage during velvet harvest and after release and reapplication of a tourniquet in 2 stags restrained in a hydraulic crush

Stag identification	1524	1524	125	125
Antler	Left	Right	Left	Right
Pedicle Circumference (mm)	154	156	162	162
Time of finishing local injections	9:50	10:02:30	10:20:20	10:41
Cut (time)	9:54	10:06:30	10:24:20	10:45
Vol local (mL)	16	16	18	18
Tourniquet tension during cut	10 kg	10 kg	10 kg	10 kg
Tourn tension when arterial haemorrhage first observed start	Full release	Full release	Full release	Full release
Tourn tension when haemorrhage ceased after re-tightening	<2 kg	<2 kg	< 2 kg	< 2 kg
Lignocaine measured in antler	0.34 mg/kg		1.1 mg/kg	

Lignocaine concentrations in the velvet antler were 0.34 and 1.1 mg/kg.

Discussion

A tourniquet applied at a tension of 10 kg prior to antler removal prevented visible arterial haem-

orrhage after antler removal. The tourniquet had to be fully removed before arterial bleeding from the cut stump was observed. After a period of bleeding, the tourniquet was re-tightened until haemorrhage stopped and the tension required to achieve this was close to 2 kg. This tension is similar to that required to prevent fluorescent microspheres and iodine from entering the antler of anaesthetised stags in Chapters 4.1. and 4.2, respectively.

This is similar to observations commonly made in the field. Typically, after tourniquet removal, there is a delay of a variable length of time before arterial spurting occurs. This haemorrhage then requires a moderate amount of pressure to stop if a tourniquet is reapplied.

This study aimed to investigate the effects of tourniquet on blood flow to the antlers in physically restrained animals that are not under the influence of general anaesthesia. It is thought the lignocaine residues reach the antler in the blood and that preventing arterial blood flow to the antler will prevent lignocaine residues. Blood flow is proportional to forward pressure and inversely proportional to resistance. Forward pressure is generated by the heart and resistance can be caused by narrowing of blood vessels, external pressure (e.g. by application of a tourniquet) or blood clot formation. It is possible that the dynamics of blood flow differs after the antler has been removed compared to the intact antler and blood vessels.

When arteries are cut, changes are induced in the vessel walls. Stretching of the smooth muscle and elastin around the vessel usually causes reflex contraction and narrowing, thus increasing vascular resistance and reducing flow. The effect of this is unknown in deer antlers.

When the vessel wall is damaged it exposes inactivated clotting factors to Tissue Factor (factor III) and triggers a clotting cascade (the extrinsic clotting pathway) that will ultimately cause clots to form around the cut end of the vessel. In the longer term contraction of the vessel combined with development of blood clots will stop bleeding from the cut surface. In the time frame that the observations reported here are made, this may not be relevant.

Bleeding observed during this study was from the cut surface of the antler rather than at the level of tourniquet application. While it is expected that adequate compression of the arteries over the surface of the pedicle would prevent obvious bleeding from the cut surface of the antler, there is little known about the course of arteries between these two structures, particularly over or through the coronet.

Applying the tourniquet at 10kg tension (well above that used clinically) did not prevent lignocaine levels above the MRL of 0.1 mg/kg in harvested antler. The two sections taken had lignocaine levels of 0.34 and 1.1 mg/kg. These results were higher than expected and may have occurred due to leakage past the tourniquet that was not observed from the cut surface of the antler.

Leakage could have occurred while the stag was moving in the crush between the time of lignocaine injection and velvet removal. The device used to tension the tourniquet was heavy and cumbersome and was fairly unstable when placed on the head of the stag. In the anaesthetised animal this was not a problem because the device could be supported and would remain completely still in any position. In Experiment 4.2, contrast iodine was seen in the antler when the tensioning device

was placed caudally on the pedicle in an attempt to avoid obscuring the view of the recordings. Positioning of the tensioning device appears to be important in the effectiveness of the tourniquet to prevent blood flow.

If high lignocaine residue occurred due to a small amount of leakage that could not be observed to the naked eye when the tourniquet was placed at 10 kg in the awake stag, it raises the question as to the significance of the small number of microspheres that were found in antlers in Experiment 1 when the tourniquet was applied. Further investigations are required to correlate antler blood flow with the amount of lignocaine that reaches the antler tissue.

An alternative explanation for the high lignocaine residues could be surface contamination of the antler during the ring block application or laboratory testing. For example, to achieve a residue level of 10mg/kg in a 10 gram sample would require $10\text{mg/kg} \times 0.01\text{kg} = 0.1\text{mg}$. As lignocaine is at a concentration of 2mg/mL, the volume of lignocaine on the surface of the section taken for analysis would be $0.1\text{mg}/2\text{mg/mL} = 0.05\text{mL}$. It is possible that this amount could be on the hands of the operator or sprayed back when injecting the lignocaine. The probability of surface contamination is increased because the sample section is close to the site of lignocaine injection.

Difficulty was experienced with adjusting the tourniquet for friction over the period of release and tightening. Because the stags were not sedated or anaesthetised it was difficult to ensure that the device stayed in the correct place and delivered a consistent pressure around the pedicle. The compression maintained by the tourniquet material even after the tensioning device was loosened probably explains why the tourniquet had to be completely removed from the pedicle before haemorrhage occurred. Haemorrhage may have been seen if the device was loosened more slowly, allowing greater time for the vessels to regain their regular shape after release of the compression.

Conclusions

Observations of haemorrhage in stags velveting using the experimental tourniquet that was used in experiment one and two concurs with previous findings that less than 2kg of tourniquet tension is sufficient to prevent arterial perfusion of the antlers.

The tourniquet device did not prevent lignocaine from entering the antler and residue levels were greater than the MRL.

4.4 Tourniquet prototype development

Introduction

Experiments in Chapters 4.1, 4.2 and 4.3 showed that a correctly applied tourniquet at a minimum tension of 2 kilograms would prevent most arterial blood flow to the antler. If the theory that prevention of blood flow will also prevent lignocaine residues is correct, then a tourniquet at 2 kg would minimise lignocaine residues in the antler. Experiments 4.1 and 4.2 showed that in some cases blood may still reach the antler when the tourniquet is in place and this may be due to gaps around the pedicle that are not completely occluded by the tourniquet.

As the antler pedicle varies in shape, size and height between stags of different age and breed, a standardised, engineered tourniquet system that can apply a consistent pressure about the antler pedicle in all stags should increase compliance and reduce the variability and failure rate of the tourniquets in current use. Desirable features of such a tourniquet are:

1. Reliable
 - a. Residues below MPL
 - b. haemostasis
2. Repeatable
3. Reproducible
4. Standardised
5. Cost effective
6. Non-painful
7. Easy to use
8. Hygienic
9. Acceptable to farmers

Current materials and methods that are used for tourniquets on farms already fulfill a number of these criteria but are not consistent and the reliability of different methods is unknown. The aim of the current experiment was to develop a system that does not detract from the positive aspects of the current systems but helps ensure standardisation.

Material and methods

An engineer in the Massey University materials testing laboratory was employed in the design of the tourniquet prototype in consultation with a physicist and past deer farmer. Further production models were then made. Several meetings were held to discuss different design options.

During the data collection for the experiment in Chapter 2, notes were taken of different tourniquet materials and methods of application. These were used as base examples during tourniquet model development.

Members of the research team attended a deer farmers' meeting in Christchurch to discuss the models that had been developed with the attending farmers.

For the purpose of model fitting and development, a section of frontal bone and attached antler regrowth was fixed in formalin for two weeks and then thoroughly washed in water. This can be seen in Figure 4.4.2, models 1, 3, 4, 5 and 7.

Results and discussion

Figure 4.4.1 shows examples of currently used tourniquet methods, as identified during data collection for Chapter 2 of this thesis, and Figure 4.4.2 shows models that were investigated during the course of this experiment.

In Figure 4.4.2, the pictures on the left hand side of the page (numbers 1, 2, 3, 4) show models that were discarded.

Item 1

This item was based on a vice grip with an adjustable tension barrel. The tourniquet was wound around the antler and the slack taken up while the jaws were open. Closing the jaws resulted in tightening of the tourniquet around a barrel. The tension on the tourniquet was maintained by friction between the barrel and the pliers and was adjusted by a nut in the centre. Problems with this model included the size and cumbersome weight, slow and difficult to put on, only one antler could be treated at one time and the friction barrel was difficult to maintain at the correct tension.

Item 2

This was a pneumatic device. A hollow rubber tubing was wrapped around the pedicle and fitted to a small compressed air canister. The slack was taken up by threading through a sliding holder and the tube was inflated. This model offered the potential advantage that the inflated rubber may conform to the irregularities on the surface of the pedicle. Problems included difficulty is making an adjustable length to fit the wide range of pedicle circumferences, relative expense and poor durability of material and inability to find suitable rubber tubing that was of small enough diameter to fit on the short pedicle of older stags, yet inflate adequately to provide sufficient pressure for haemostasis. The tubing needed to be no more than 5mm in diameter and of relatively dense, durable rubber. This resulted in a small inflation volume and inadequate pressure. Item 2b shows alternative arrangements for adjusting the length of the tourniquet.

Item 3

This was a cable tie applicator with adjustable tension and cutter. The cable tie was placed around the pedicle, threaded through the fastener and the long end as placed in the device. The lever was used to pull the cable tie up to the desired tension at which point a cutter was activated and thus releases the cable tie and prevents over-tightening. Problems with this method included non-reusable materials (although releasable ties were investigated), only one antler could be dealt with at a time, difficulty in getting the device close up to the pedicle to assure adequate and even tension.

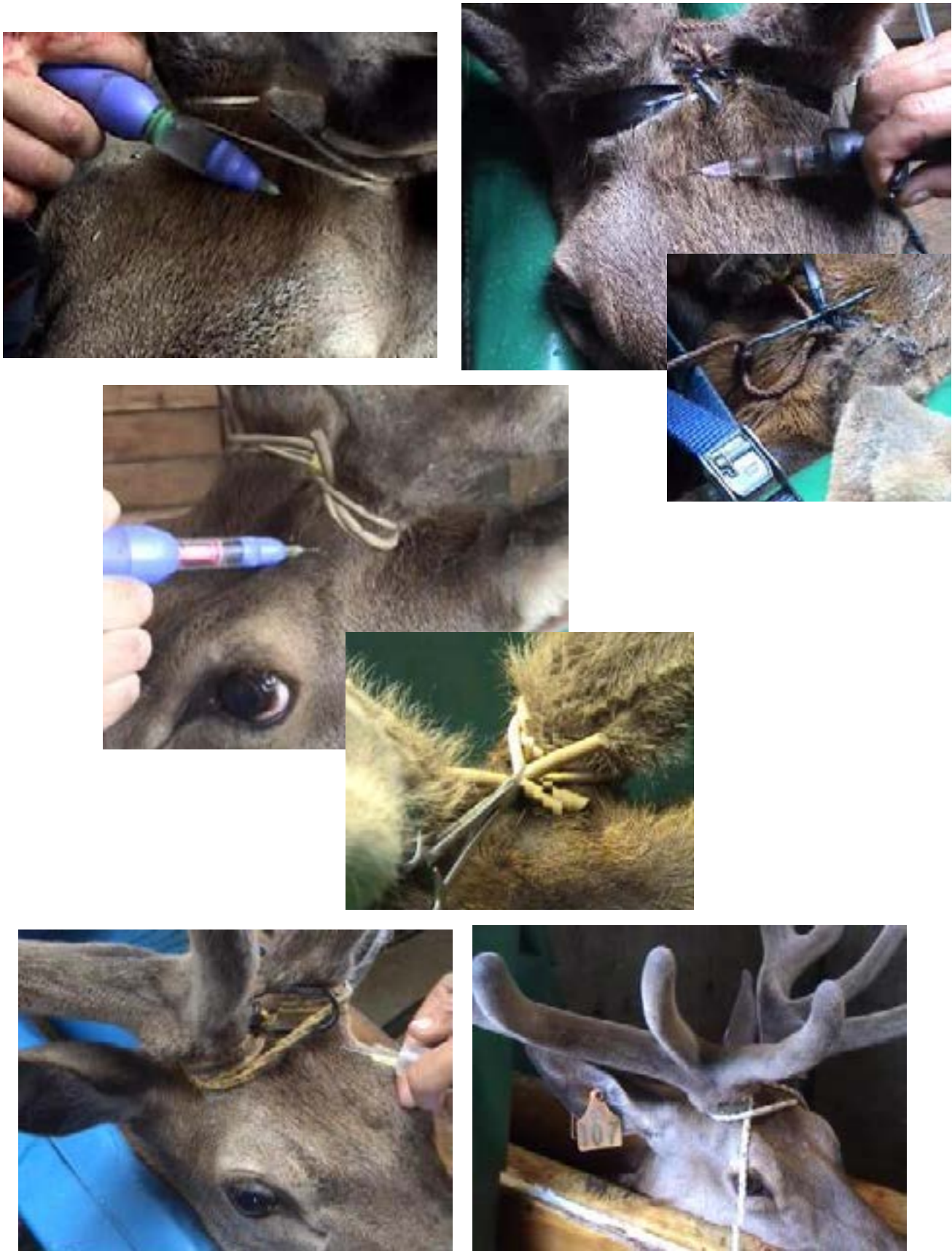


Figure 4.4.1 Examples of tourniquets used in the field. Clockwise from top left: Rubber band, Car inner tubing (inset fastening pin); Solid rubber cord (inset fastened with a haemostat) advocated by NVSB; Rope attached to the crush on both sides; Bungy tie down.

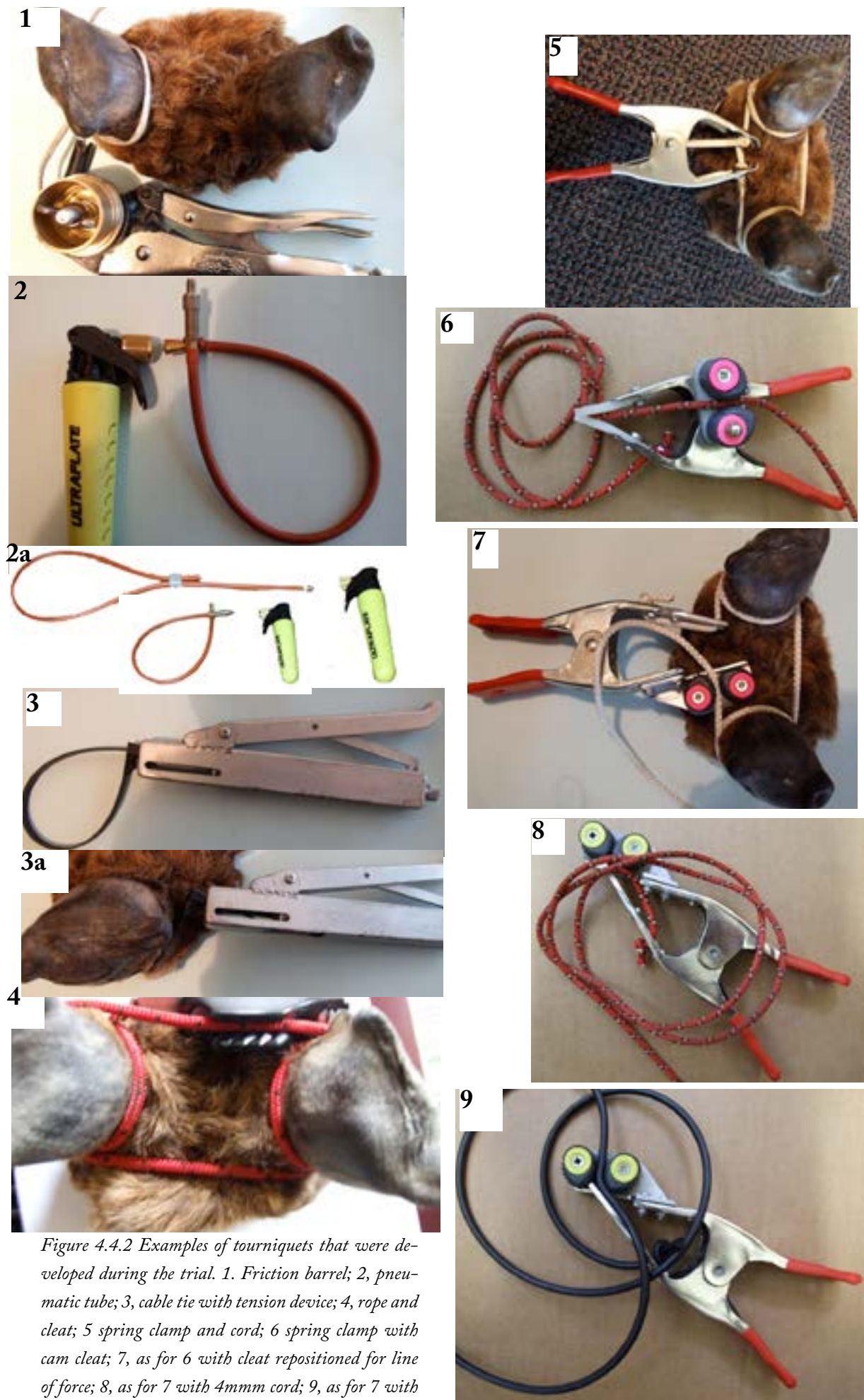


Figure 4.4.2 Examples of tourniquets that were developed during the trial. 1. Friction barrel; 2, pneumatic tube; 3, cable tie with tension device; 4, rope and cleat; 5 spring clamp and cord; 6 spring clamp with cam cleat; 7, as for 6 with cleat repositioned for line of force; 8, as for 7 with 4mm cord; 9, as for 7 with solid rubber O ring cord.

Item 4

This was a piece of plain cord held in place using a marine cleat. The main purpose of this model was to experiment with a different method of holding the ends of the cord in place. The cleat is small, unobtrusive, lightweight and durable. It did not reliably hold the ends of the rope in place and tended to twist when applied at an angle.

Item 5

For this model, the cord was held with a spring clamp. The tourniquet was wrapped in a single complete circle around one antler, straight across the front or back and then in a complete circle around the second antler. The tension was held at the correct level by the spring clamp which delivered a pull force of 6kg provided that the jaws of the clamp were not touching. This model was quick to apply and had the added advantage of treating both antlers at once. The tension applied on the tourniquet could theoretically be altered by placing a softer or firmer spring in the clamp. The first prototype was not easily adjustable with set lengths of tourniquet required for different antler diameters. There was some room for variability within the range of the jaw opening.

Item 6

This was a development of item 5 to overcome the need for variable tourniquet length. Here a cam cleat was fitted to the clamp and the free end of the tourniquet was pulled through this after wrapping around the antler. The placement of this cleat was problematic because the direction of the force applied by the tourniquet caused the device to twist after application.

Item 7

This was a modification of 6 but with the cam cleat placed in line with the pull direction of the tourniquet material. This resulted in the tourniquet sitting quite stable against the head or neck of the stag. Advantages of this model included quick application from either the front or back of the stag, re-usable material, quick application and quick release. The tool was also easy to use for females with smaller hands and a weaker grip and for farmers with large hands because it did not involve fiddly fittings or ties. Potential problems were the large and relatively obtrusive nature of the clamp that the stag may not accept and potential slippage in the cam cleat. It was thought that these potential problems could be overcome by streamlining the model. This final model was the device that was taken for field testing.

Items 8 and 9

Two different models were used for the field testing as the type of material used in the tourniquet may affect its operation. One model was fitted with a non-elastic 4mm shock cord (item 8). The second model was fitted with 4mm dense rubber that is used for manufacture of O rings (item 9). This had moderate elasticity and was thought to be durable and easily cleaned.

Several models and potential methods were discussed and created. Most of these were not suitable as they did not fulfil the criteria. The ultimate testing of the tourniquet is whether it

can prevent excessive lignocaine residues in harvested velvet. The cost of lignocaine testing was approximately \$200/sample. Only a small number of tourniquet models could be tested for their ability to prevent lignocaine residues within the finances available. The final models 6, 8 and 9 were chosen for preliminary field testing (Chapter 4.5).

Conclusion

Developing an ideal tourniquet system that fitted all the desirable criteria proved to be challenging.

4.5: Field testing of tourniquet models stage 1

Introduction

Feedback from farmers at conferences and meetings indicated that there was a mixture of scepticism and support for the tourniquet system. Many felt that they would not judge until seeing how it worked on the animals. This experiment was the first testing of the tourniquet in an on-farm velveting situation. The aim was to determine how the prototype tourniquets performed on farmer acceptance, stag acceptance, ease of application and removal, haemostasis and stability on a range of different farms and with different operators.

Materials and methods

The test tourniquet was made in 12 copies, Five of model 8 and five of model 9 and 2 of model 6 developed in the experiment in Chapter 4.4 (Fig 4.4.2).

Ninety five stags, four years and older, on eight different properties were velveted using these models. Eleven farmers or assistants were invited to use the tourniquet. Four of these properties were in the South Island and four were in the North Island. Three velveted stags under standing sedation, four velveting using local only and a crush for restraint and one used a combination of these methods.

On six of the eight properties, video recordings were made as follows.

- A wide angle camera was set at a distance from the crush and used to video the stags whole body movements and application of the tourniquet.
- A close up, hand held camera was operated during velvet removal only and footage used to observe tourniquet stability and degree of haemorrhage after antler removal.

Ten randomly selected samples of velvet antler (4 removed using model 8 and 6 removed using model 9) were analysed for lignocaine levels at Agriquality Ltd, Huarangi, Wellington using Gas Chromatography and Mass Spectrometry. For this analysis, a slice was removed from the base of the antler as specified by the NZFSA. Spiker antler was removed using *NaturO*TM rings and this was supplied to the laboratory as a blank sample.

Results

Full details of observations are shown in Table 4.5.2 and are summarised below in Table 4.5.1

Table 4.5.1 Mean and range of factors and comments recorded during the first stage of tourniquet prototype testing on adult stags during velvet antler removal

Factor	Mean	Range
Age of stags	6.4 years	4 to 12 years
Pedicle diameter	180mm	140-235mm
Local per antler	17mL	12-25mL
Time to apply tourniquet	20 seconds	
Time to remove tourniquet	3.5 seconds	
Stability/durability	No breaks or displacement Model 6 twisting/weakening	
Haemorrhage	Degree of haemorrhage that occurred while the antler was being cut was recorded and is presented in figure 4.5.1	
Other comments recorded	Most farmers commented “not very tight” vModel 8 had a tendency to twist due to the offset cleat. Model 9 did not twist. Some profuse bleeding was seen after tourniquet removal Rubber prototype was preferred by farmers	

Lignocaine results

10 samples were tested for lignocaine residue levels. The results are shown in the top panel of Table 4.5.3. Also shown in Table 4.5.3 are results from previous studies for comparison. These are discussed further in the Introduction (Chapter 1.4)

For the Rope prototype (Model 8, Chapter 4.4), 4 samples were tested and the results were 0.41, 0.67, 1.3 and 6.44 mg/kg lignocaine. All of these were above the MPRL of 0.1mg/kg.

For the rubber prototype (Model 9, Chapter 4.4), 6 samples were tested and the results were 0.03, 0.04, 0.07, 0.07, 0.08 and 0.51 mg/kg lignocaine. 5 out of 6 of these were below the MPRL.

Haemorrhage score.

Figure 4.5.1 shows the number of antlers with each haemorrhage score (0 to 5, where 0 represents no haemorrhage and 5 arterial spurting). Within each haemorrhage score, the number of antlers removed with each of the 3 tourniquet modes is shown

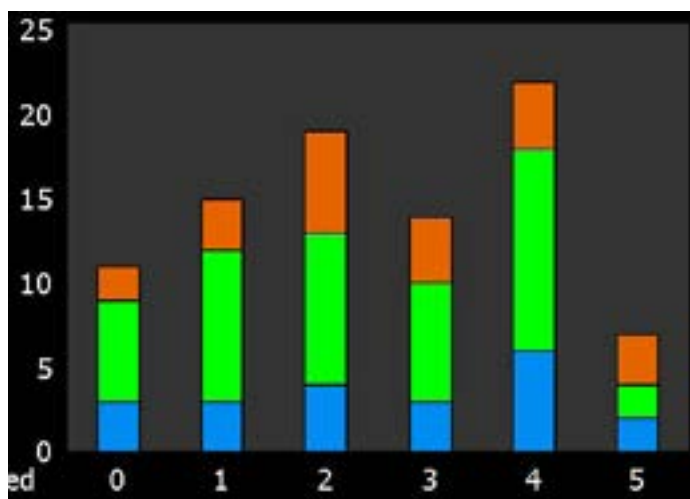


Figure 4.5.1 Number of adult stags with each haemorrhage score recorded during cutting the velvet antler during the first stage of tourniquet prototype testing in the field.

Red: Rope material, offset cleat, Green: Rope material, (model 8), Blue, Rubber (model 9); Score 0= dry, 1= slight ooze, 2=slow drips, 3= fast drips, 4= constant stream, 5= spurting

Table 4.5.2 Details of observations during testing of two prototype tourniquets on farms during normal velvet harvest in adult stags four years and older.

ID	Tourniquet	Application	Video
Blue 904 sedated	new/rubber	Big old stag large antlers.	TQ1 00:07:00
lost tag sedated	new/rope	Old stag, easy application, gap between jaws	00:13:17
Pink 35 sedated	new/rope	Difficulty getting tourniquet on. Sat well, then left rope slipped out of guide. Thick around pedicle. Not touching 2nd time	00:54:12 to 2:09:16.
yellow 40 sedated	new/rubber	Slipped out of guide when applying. not touching when first put on. 15secs	02:14:11
Pink 44 sedated	new/rubber		08:34:20, 08:54:08
Yellow 83 sedated	new/rubber		no vid
904 sedated	new/rope		no vid
85 sedated	new/rope		no vid
Pink 41 sedated	new/rubber		13:33:15
??15 sedated	new/rope		13:40:09, 14:02:10
14 sedated	new/rubber		14:51:10, 15:25:19
465 sedated	new/rubber		no vid
26 sedated	new/rubber	A bit fiddly but less than 30 secs	17:58:15, 18:34:16
93 sedated	new/rope	about 10 secs, good gap	18:54:05, 19:39:08
old B 274 sedated	new/rope	2 attempts.	19:59:04, 21:12:22
yellow 6 sedated	new/rubber		21:12:22, 22:14:18
99/54	new/rope		RB12: 2:12:11, 2:43:14
02./61	new/rope	Easy on, sitting well, jaws well apart. Top up of local left antler	RB12: 14:15:21, 14:41:04
02/130	new/rubber	Easy on, but probably not pulled tight enough at start.	RB12: 21:37:11, 21:57:01
01./62	new/rope	10 secs to put on. Jaws well apart	RB12: 30:13:04, 30:23:02
99/55	new/rope	approx 15secs. Jaws seem a littl etoo close when first put on. Pulled rope tight during application.	RB12: 35:44:16, 36:09:17
02./60	new/rope	stag jumping up wall before start. Quick on. Jaws apart when applied 28mL/side. 1120mg 4-5mg/kg	RB12: 41:34:22, 41:52:21
94/167	new/rope	not much force on sting when wrapping around pedicle.	RB12: 46:16:01, 46:32:15

Cutting	video	Operator (local)	result mg/ kg
Spurting lots arterial blood. Right antler. Although jaws still not touching	2:45:08, 3:50:00	Farmer	0.67
Jaws very close during cut. No bleeding.	3:52:10, 4:55:00	Farmer	
Sticking up on funny angle, rotated, jaws touching, Spurter on left	4:58:12, 6:13:18	Farmer	
Jaws touching but no bleeding	6:14:24, 7:22:10	Farmer	
Jaws may be touching. Some bleeding, oozing	9:55:00, 11:00:00	Farmer	0.07
No bleeding, jaws touching	09:01:24, 09:52:18	Pania	
Jaws touching? Maybe not. Completely white surface on cut	11:07:17, 11:46:03		
Jaws not touching. Completely bloodless	11:56:07, 12:39:10		
	16:00:21, 16:51:05	Farmer 28mL local	0.07
	17:22:22, 17:55:00	Farmer	
No bleeding	15:26:19, 15:57:02		
Was bleeding a bit but hard to say if from outside or middle	22:19:20, 22:53:01	Farmer	
completely dry	22:54:01, 23:27:24	farmer 12-14mL per side	0.07
bleeding quite a bit although jaws don't appear to touch	23:30:18, 24:51:23		
completely dry and jaws not touching	24:51:23, 25:23:03		
Dripping quite a lot of blood jaws very close or touching	RB12: 9:49:24, 10:53:18	Pania 20-24mL local	
Jaws still well apart	RB9: 13:04:15, 14:11:21		1.3
Quite a lot of dripping off back of antler	RB12: 19:11:12, 20:10:07	Farmer, Pania lots of local	
Jaws come together quite slack in rubber.	RB12: 26:28:19, 28:18:12	Pania	
Lots of blood	RB9: 15:13:07, 16:05:00	lots local	
Jaws still well apart even though jumped around a lot.	RB9: 16:06:24, 17:48:24	Pania	1.3
Quite bit of blood during cut from sut surface. Fidgety	RB12: 32:57:00, 34:32:21	lots local	
jaws still apart, although not particularly wide when applied. Dripping moderately	RB12 37:57:22, 39:23:13	Farmer	
Jaws still apart. Antler looks pretty dry from above.	RB9 17:57:01, 19:27:00	Pania lots local	
Cut on bit of a funny angle, but not much blood	RB12: 44:33:03, 44:38:01	Pania	1.3
Top up local left. Jaws may be just touching. Stag	RB9: 19:29:20, 20:26:01	lots local	
struggling. a lot of bleeding	RB 12: 48:36:03, 52:37:04	Pania	
	RB9: 20:28:13, 21:51:23		

ID	Tourniquet	Application	Video
02//54	new/rope	Quick on. Pulled up a bit better, jaws wider	RB12: 52:24:22, 53:34:09
01/411	new/rope	Easy on, sitting jaws apart but not much pressure applied during application	RB12: 57:34:22, 57:48:14
02./54	new/rope	A bit tighter when pulling up	RB12: 1:04:51:07, 1:05:05:07
98/99	new/rubber	Not sitting well. Too far to the right. Jaws a bit close.	RB12: 1:09:44:13, 1:10:12:10
01./39	rubber inner tube	About 15secs	RB12: 1:16:51:03, 1:17:06:10
01./1	rubber inner tube		RB12: 1:24:37:23, 1:24:49:14
99/251	rubber inner tube		RB12: 1:31:57:15, 1:32:34:02
99/327	rubber inner tube		No tape
97/231	new/rope	Lots of local (30mL/side)	RB13: 2:18:16
00/34	new/rope		RB13: 06:57:07, 07:07:16
99/144	new/rope	Easy on, sitting well, looks fairly firm	RB13: 12:44:22, 13:00:24
sedated 01//30	new/rope		Not on film
sedated 683	new/rope		RB10: 37:54:15, 38:32:18
00/356	new/rope		RB10: 45:24:14, 45:50:11
02/1521	new/rubber	Struggled during application, but still took 20secs	RB10: 51:30:05, 51:52:00
02/085	new/rubber		RB10: 58:00:01, 58:27:05
02/075	new/rubber	a bit tricky, needed a second attempt to get on properly. Not wrapping the right way.	RB10: 1:04:38:14, 1:05:30:12
01/1055	new/cord	Much quicker and easier now he's got the hang of it. Stag jumping around a lot during local and after.	RB10: 1:11:31:17, 1:11:48:15
99/530	new/rubber	Took a long time to put it on	RB10: 1:17:20:10, 1:18:53:23
01/1337	new/rope	This farmer found technique more difficult to grasp	RB10: 1:24:03:04, 1:24:39:09
00/226	new/rubber		not on tape
99/232	new/rubber		not on tape

Cutting	video	Operator (local)	result mg/kg
Jaws still well apart, hardly any blood-slow drips.	RB12 55:35:07, 56:08:22	Pania	
	RB9 21:52:23, 22:41:23		
Bleeding quite a lot, Jaws may be touching	RB12 1:01:26:01, 1:03:18:09	Pania	
	RB9: 00:01:00, 00:01:37:12		
Jaws well apart. Few small drips of blood	RB12 1:06:57:12, 1:07:50:15	Pania	
	RB9: 01:39:20, 02:37:24		
Slipped around further to the right.	RB12 1:12:47:03, 1:14:20:10	Pania	
	RB9: 02:42:01, 04:06:15		
Completely dry	RB 12: 1:20:05:24, 1:21:31:03	Farmer	
	RB9: 04:08:16, 05:32:03		
Sample taken	RB12 1:27:00:01, 1:29:53:13	Farmer	
A few small drips only	RB12: tape end	Farmer	
Slow drips. Coagulating on stump	RB9: 05:33:17, 07:01:18		
	RB13: 00:12:10, 01:48:05	Farmer	
Twisted, not sitting that well. Jaws well apart. Completely dry. Farmer comment "that's perfect - on all counts"	RB13: 05:00:10, 05:33:23	Pania	0.41
A few drips of blood. Jaws pretty close together.	RB9: 07:07:04, 07:44:04		
	RB13: 10:04:02, 10:32:03		
	RB9: 07:45:14, 08:12:22		
Took long time to cut, Jaws still apart No bleeding	RB13: 15:38:06, 17:15:24		
	RB9: 8:14:16		
Tourniquet looks fine, but really dopey stag.	RB9: 9:32:15, 9:55:19		6.44
A little bleeding. Tourn looks to be holding OK.	RB10:43:31:04, 43:51:04	Pania: Lots local, P's	
	RB9 86min 8:24:20, 8:43:01	ring block	
Moderate bleeding	RB10:49:06:19, 49:38:06	Farmer	
	RB9: 8:45:15, 9:08:13		
Quick cuts. Jaws apart dripping a bit.	RB10: 55:45:16, 56:11:12	Pania: 20-24mL local	0.04
	RB9: 9:10:05, 9:37:16	per side	
Jaws well apart still, Basically no bleeding.	RB10: 1:02:03:21, 1:02:43:19	Farmer	
	RB9: 09:40:09, 10:23:24		
Jaws well apart, lots bleeding "Good local though" "yeah but in terms of the tourniquet.. well.. I think we have a few improvements to make"	RB10: 1:08:41:23, 1:09:18:23	Farmer	
Stag climbing up the wall before cut, no movements during cut. A little bleeding	RB9: 10:24:22, 10:59:17		
	RB10 1:15:00:06, 1:15:26:14	Farmer	
	RB9: 11:01:00, 11:22:00		
Struggling and growling lots during wait time but not during cutting. Moderate bleed	RB10 1:22:53:24, 1:23:13:08	Farmer B	0.03
	RB9 11:24:00		
Heaps bleeding but not spurting.	RB10 1:28:31:21 1:28:49:04	Farmer B	
	RB9 11:47:12, 12:01:15		
A little bleed but not much.	RB9: 12:03:03, 12:33:12		0.51
Completely dry	RB9: 12:34:02, 12:52:08		0.08

Discussion

The models tested proved to be well tolerated by stags both sedated and restrained in the crush. On some occasions, the clamp was observed to stick out at an angle from the head. Bumping of the clamp was an issue if the stag became recumbent. The shape and size of the clamp should be refined to make it smaller and less likely to be knocked. Also, the design needs to be modified so that it fits flat against the head of the stag in all cases.

Farmers found the device easy to apply after learning the correct technique and application to both antlers took an average of 20 seconds. The biggest advantage was seen in the quick and easy removal. This was easiest in models that had a shorter length of nonelastic tourniquet material and was especially useful in standing sedated stags as it was a simple matter of grasping the free end and pulling it upwards out of the cam cleat.

Most farmers commented that the tourniquet was not very tight compared to the methods they were used to. In some cases haemorrhage was quite pronounced with a constant stream of blood flowing from the cut surface. Haemorrhage was reportedly greater using these models than with their standard methods and the degree of tightness may be related to the amount of haemorrhage. If that is the case it would be expected that local anaesthetic would be able to enter the antler when a greater degree of haemorrhage was seen. There was no apparent relationship between the degree of haemorrhage and lignocaine residue in the antler. The stag with the lowest residue (0.03mg/kg) was noted to have moderate bleeding during cutting and the deer with the two highest levels of lignocaine had very little bleeding during cutting. One stag with spurting arterial blood had a residue level of 0.67mg/kg which was greater than the MRL, but not excessively high compared to other values in this and previous studies.

It was often not possible to tell whether the blood was flowing from the centre of the antler or the periphery and whether it was venous back flow or in fact arterial blood.

The rubber cord was preferred by most farmers, probably because it was more similar to a material that they are used to working with. Despite this the rope cord was slightly easier to apply and tighten.

Lignocaine results were higher than anticipated. There appeared to be a difference in the rate of compliance dependant on whether the rope or the rubber material was used. The rubber prototype returned five of six results below the MRL and the sixth was 0.51mg/kg. The rope prototype resulted in lignocaine levels that were all in excess of the MRL of 0.1mg/kg and one result was 6.44 mg/kg, more than 60 times greater than the MRL.

The difference between the rope and rubber prototypes in mean lignocaine residues was not statistically significant ($p=0.104$) using non-parametric one-way ANOVA with unequal variance. This result is most likely to be due to small numbers in the groups. This is a concern and suggests that this tourniquet is not achieving its purpose.

Conclusions

The concept of the current model was well accepted by farmers and it is easy to use. Modifications are required to achieve better haemostasis and compliance with lignocaine residues.

The rubber material achieved better residue compliance thus this should be the starting point for future development of the tool.

Possible modifications include increased tension, elastic or rubber material and streamlining and decreasing the size of the clamp. This study is presented in Experiment 4.7.

4.6: Studies on the anatomy and arterial pattern of the pedicle and antler using acrylic arterial casting and antler pedicle sections

Introduction

It is thought that lignocaine residues reach the antler in the blood supply and that noncompliant levels are most likely to be due to failure to fully occlude the arterial vessels supplying the antler. Adams (1979) described the vasculature and nerve anatomy of the antler pedicle based on dissections, angiograms and latex casts of heads of stags. Those dissections showed that the arterial supply was from branches of the superficial temporal artery. The first branch coursed caudolaterally and supplied the caudal and caudo-medial regions of the pedicle. The second branch was smaller and supplied the lateral side of the pedicle and the third branch continued around the rostral and rostromedial regions of the pedicle. From these three main branches forming a ring around the base of the pedicle, 9-12 sub-branches were described passing dorsally up the pedicle itself. Anastomoses were not seen with the naked eye and the blood supply beyond the pedicle was not described. It would be useful to have a more precise model of the arterial supply to the antler, particularly at the level of the tourniquet so that a tourniquet model to account for the anatomy or the failure of tourniquets to prevent lignocaine residues might be explained.

This study aimed to produce a model of the arterial supply to the antler, using intra-arterial methyl methacrylate casting material. This would allow visualisation of the entire superficial arterial supply to the pedicle and antlers from the level of the external carotid artery to the tips of the intact antlers in an adult stag. In the second part of this experiment, antler pedicles were sectioned at the level of anticipated tourniquet placement for visualisation of the contour of the pedicle.

Materials and methods

Acrylic Cast

One mature stag with antlers developed to the stage for normal commercial harvest was purchased from a deer farm for this study. The stag was purchased before antler growth commenced and was kept on the Massey University Deer Research farm until the date of this study.

The animal was anaesthetised with intravenous propofol and ketamine as for the experiments in Chapters 4.1 and 4.2. On induction, the animal was intubated and transported in a horse float to the Massey University Veterinary Teaching Hospital postmortem facility.

On arrival, the animal was connected to an anaesthetic machine and anaesthesia was maintained with halothane delivered in oxygen. The anaesthetised animal was given heparin (25000 units/ml, Leo Pharmaceutical Products Ltd, Ballerup, Denmark) administered by the intravenous route, at

1000 units/kg of body weight, to prevent the development of blood clots post-mortem (Tompsett & Wyke, 1969). The heparin was allowed to circulate for approximately two minutes before the animal was euthanased by an overdose of anaesthetic. (20% pentobarbitone sodium; Boehringer Ingelheim Pty Ltd, Artarmon, Australia)

The head and neck of the carcass was removed as a unit from the remainder of the body by severance of tissues through a transverse plane in the mid-cervical region. The carotid arteries were isolated and cannulated for introduction of the acrylic casting agent. Residual blood was flushed out using heparinised saline. The specimen was then secured in a metal frame to stabilise it against fracture of cast vessels during later handling.

Methyl methacrylate (Aldrich Chemical Company Inc.) was infused into the common carotid arteries through cannulae connected to a pressure pot containing the acrylic medium, and driven by compressed air.

The infusion pressure was monitored using a pressure gauge on the output side of the pressure pot and the rate of delivery of the acrylic was adjusted to ensure that the maximum pressure did not exceed 100 mm Hg.

The infusion was judged to be complete when red acrylic leaked from small incisions made into the velvet at the tips of the antler.

A colony of dermestid beetles was used to remove the bulk of soft tissues to expose the cast. This process took several months, and afterwards remnants of soft tissue were cleared by immersing the specimen in 5% potassium hydroxide solution, followed by careful rinsing in tap water and drying in air.

Pedicle sections

Ten stag heads were collected from the Fielding Deer Slaughter Premises. These were frozen and the top portion of the head and antlers removed for study. The pedicles were sawn at the approximate site of tourniquet application. Photographs were taken and measurements made of the circumference, length and width of the pedicle.

Adobe Illustrator live trace software was used to outline the surface of the cut pedicle in order to visualise the relief pattern at the point of tourniquet contact.

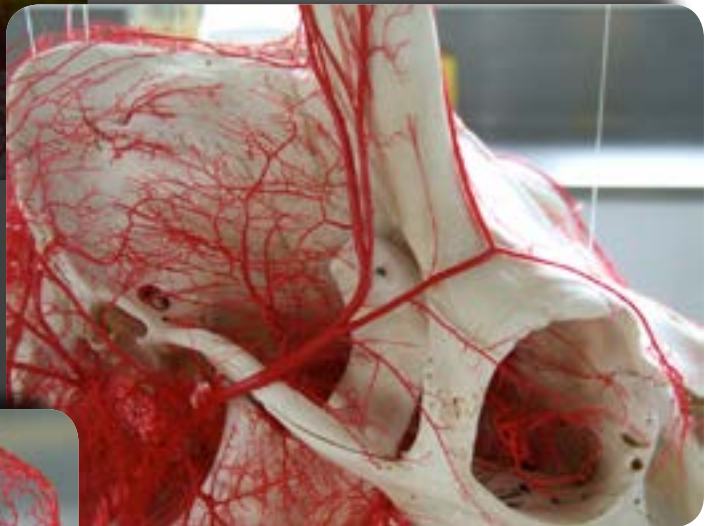
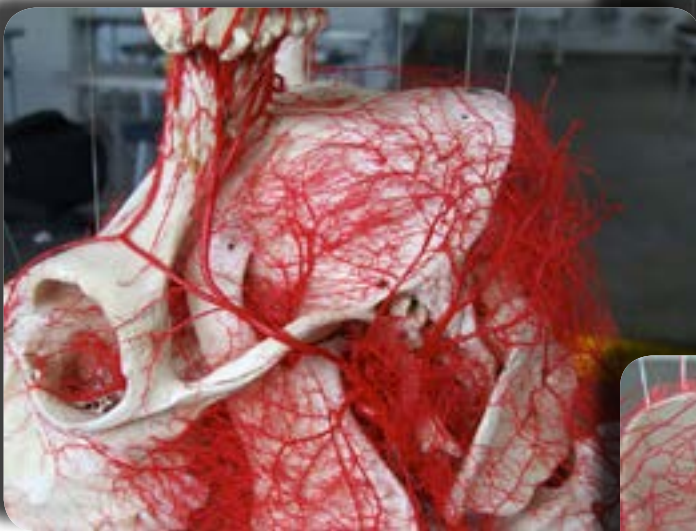
Results

Photographs of the methyl methacrylate cast are shown in Figure 4.6.1. Photos of the cut sections of pedicle are shown in Figure 4.6.2 and outline tracings using Adobe Illustrator live trace are shown in Figure 4.6.3 and an enlarged view in Figure 4.6.4

An acrylic cast of the arteries on the exterior part of the skull and antlers, with the bone portions of these tissues remaining intact, was successfully created. The left antler created a better cast than



All arteries could be traced back to the superficial temporal artery crossing the zygomatic arch



Anastomoses at the tip of the antler



Figure 4.6.1 Photographs of an acrylic cast of the arterial supply from the carotid artery to the antlers and head of a mature male red deer.



Coronet. Casting of the antler occurs below this point. Arteries cross the coronet between ridges in the outer edge and through holes located closer to the antler.





Figure 4.6.2 Transverse section cut through the antler pedicle of adult stag heads at the level of tourniquet placement showing outline of the skin and location of bony core within the pedicle.

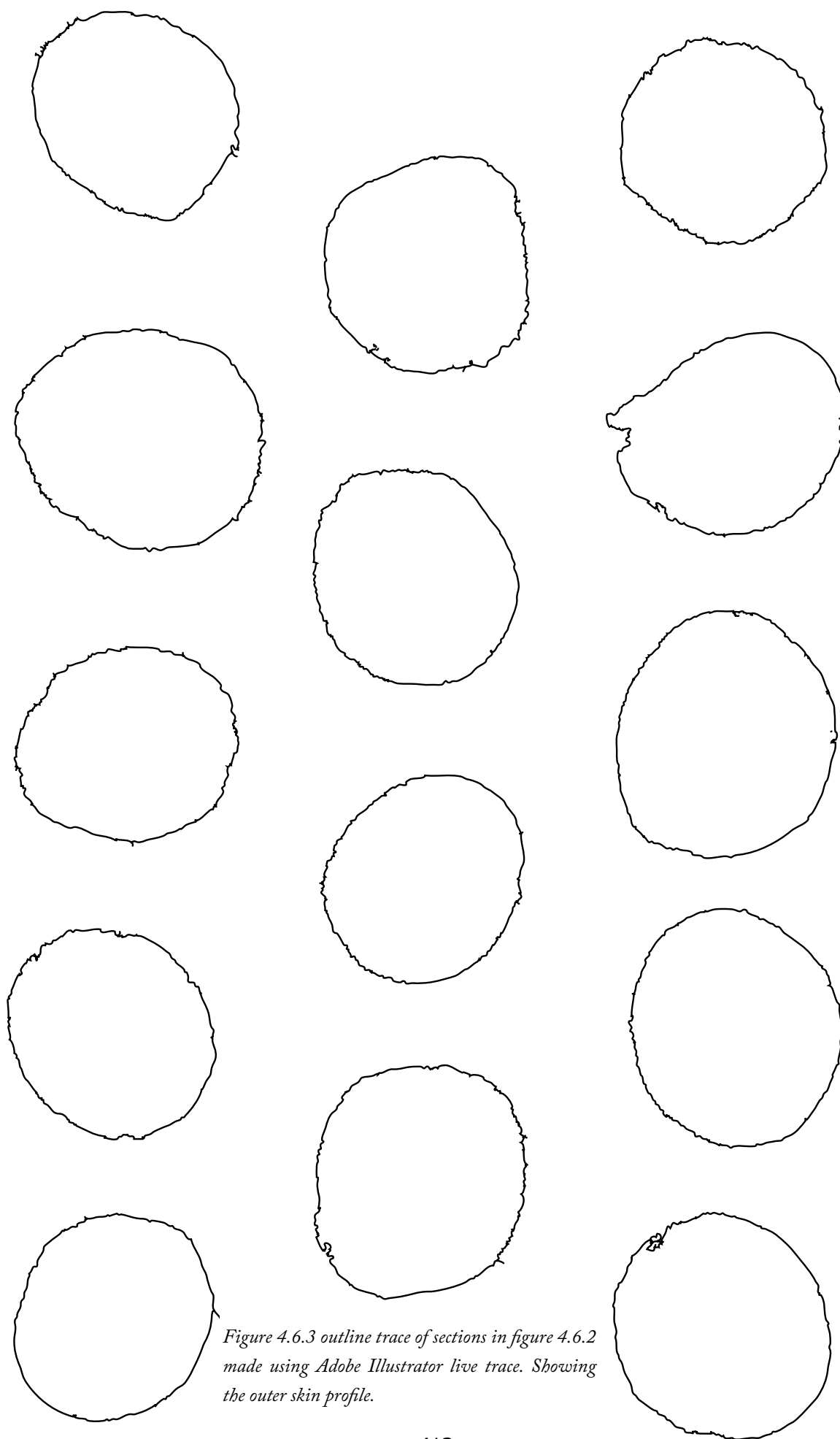


Figure 4.6.3 outline trace of sections in figure 4.6.2 made using Adobe Illustrator live trace. Showing the outer skin profile.

the right antler. The cast revealed an elaborate and tortuous network of arteries crossing the antler pedicle and travelling between and through small channels at the junction of the antler and the pedicle. There were numerous anastomoses and vessels were seen to overlay each other in several places. The superficial temporal artery was the sole artery supplying the antler distal to the external carotid artery that was observed in the cast.

Cross sections of the pedicle revealed variable and irregular sizes and shapes at the expected level of tourniquet application. The pedicles were not consistently circular or oval in shape and numerous small irregularities including small depressions were apparent in the skin, subcutis and underlying bone. The outline of the bone was generally smoother than that of the skin. The overlying dermis and subdermis was of variable thickness and contained numerous small and large blood vessels that were not preserved adequately during sectioning to allow close examination. The bony core was made of a porous structure containing blood vessels in the centre.

Discussion

The acrylic cast resulted in well demarcated elaborate arterial preservation over much of the head, neck, pedicle and antlers. Some regions did not cast well and this may have been due to pressure on regions of the head, neck or antler during anaesthesia and transport of the stag.

The arterial pattern seen in this cast is similar the finding of Adams (1979) in that the arterial supply appears to come entirely from the superficial temporal artery and the branching pattern seen here is similar to that described, although the proposed “circle” around the pedicle is less clear in our model. Unlike the observations recorded by Adams, numerous anastomoses can be seen

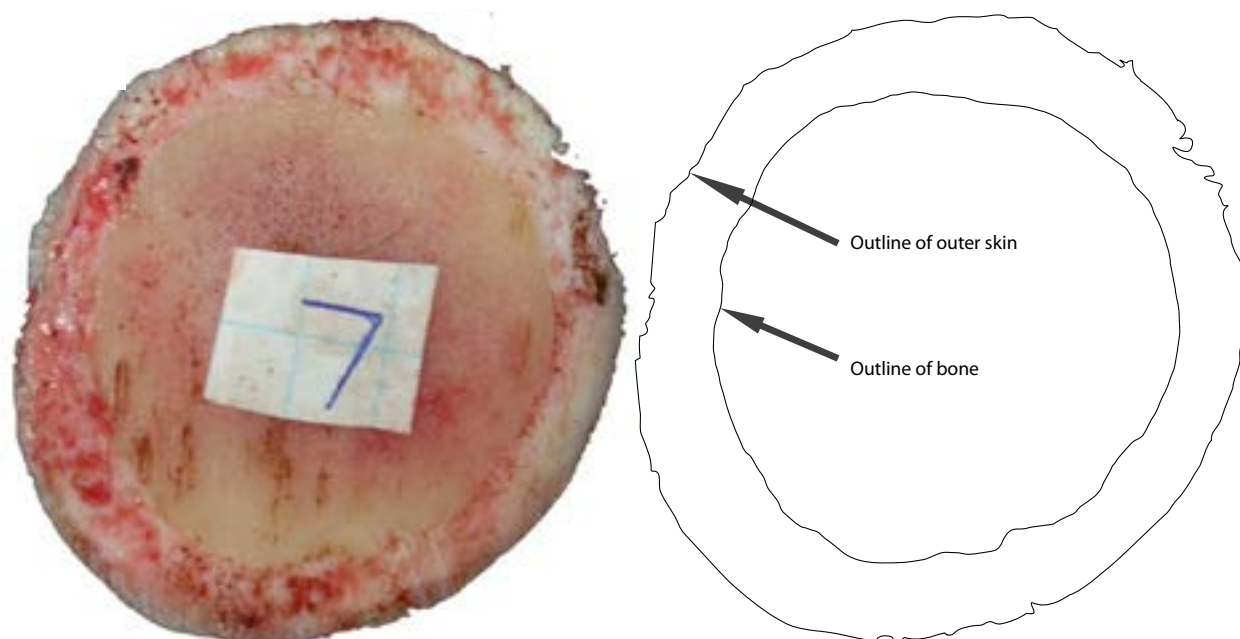


Figure 4.6.4 Larger view of the transverse section of an antler pedicle of a mature red deer stag (Antler 7, Fig 4.6.1) and relief outline showing the outer skin and the outline of the bone of the pedicle. Numerous blood vessels are present in the dermis and subdermis and a trabecular pattern is evident in the centre of the pedicle bone.

crossing the surface of the pedicle. Furthermore, we observed considerably more than the 9 to 12 smaller branches crossing the surface of the pedicle that have been previously described and in some places arteries were observed overlying each other, particularly on the caudal aspect of the pedicle (Figure 4.6.1).

The coronet of the antler (junction between the antler pedicle and antler itself) was seen as an irregular, knobbly structure with several complete holes around the outer rim. These holes provided for the passage of arteries that crossed onto the antler and continued along its superficial surface unhindered. This explains how the blood vessels and nerves may be located superficially on the pedicle and cross the seemingly tortuous route across the coronet onto the antler. This is also a likely conduit for lignocaine residue to reach the antler itself. The coronet is easily palpable as an irregular bony structure in the live animal and this part is cast each year with the antler or remaining button if the stag was velveted. A new coronet grows each year along with the antler, blood vessels and nerves.

Cross sections of the pedicle reveal large variation in the shape and size of the pedicle. There are also irregularities observed in the shape and thickness of the dermis covering the pedicle and in the bony structure. Numerous vessels are seen deep within the outer fibrous dermis layer and the irregular nature of these suggests that it may be difficult to achieve complete occlusion in all cases, using a tourniquet. It can not be determined from these sections whether these vessels are arterial or venous in nature. Given the close proximity of the arterial supply with the venous drainage in other regions of the antler it is reasonable to assume that they are a combination of the two.

The tracing outlines shown in Fig 4.6.3 give an indication of the variation in shape and surface structure of the outer pedicle surface. Some of the irregularities are likely to be artefact. For example antler 9, second from the top on the far right fractured while being cut and a piece of pedicle appears to protrude out from the upper left corner. Where there was overlap of hair across the antler surface, the software tending to create more indentations into the outer surface than were actually due to surface irregularity.

Closer observation of the cut sections in Figure 4.6.4 shows that the bony structure within the pedicle is not always the same shape as the outer skin surface. The bony outline tended to be smoother but still showed variation between antlers, even within the same animal and in some cases was quite irregular in shape.

Conclusions

Acrylic arterial casting of the arterial supply to the pedicles and antlers revealed an elaborate and vast vascular supply with numerous branches, anastomoses, overlapping of vessels and passage across the coronet onto the antler. Cut sections of antler revealed irregularities in both the skin surface and the inner bony structure of the pedicle with vascular regions varying in depth and thickness within the deep dermal layer.

These findings suggest that the anatomy of the pedicle and vascular supply may account for the failure of tourniquets to fully prevent blood flow and thus lignocaine residues to the antlers.

4.7 Lignocaine residues from harvested velvet when different tourniquets were used for haemostasis

Introduction

Previous experiments described in this chapter showed that, in anaesthetised deer, a model tourniquet applied at a tension of 2 kg was sufficient to prevent most blood flow to the antler. This was demonstrated by the absence of fluorescent microspheres and contrast iodine in the antler in Chapters 4.1 and 4.2 respectively. Prototype tourniquet development led to two models being field tested during the 2006/2007 velvet season. Random antler sections were taken and tested for lignocaine residue (Chapter 4.5). One model (Model 9) resulted in 5 of 6 randomly selected antlers with lignocaine residues less than 0.1mg/kg. In the final testing stage in this experiment, we started with Model 9 and made modifications in response to antler residue results. The aim was to refine the tourniquet to a system that would be reliable and consistently effective at preventing noncompliant lignocaine residues.

Materials and methods

Eighty nine antlers from 45 stags were used in this study. Of these, 74 had samples submitted for lignocaine residue testing. These experiments were conducted at two properties in the Hawke's Bay. Only stags 3-years and older with well developed antlers at the correct stage of growth for commercial harvest were accepted for the study. The stags on one property were velveted by the owner and the stags on the other property were velveted by a veterinarian.

The original planned methodology was to test ten samples taken using one tourniquet and then modify this as required until results were within an acceptable range.

As the turnaround time between sending samples to the laboratory and receiving the results was estimated to be ten working days, it was not practical within the tight time frame of one velvet season to wait for the results from one batch of tests before making modifications and proceeding with the next.

On each study day the stags were brought into the yards and treated as follows:

1. Stags were sorted into pens of three to four stags per pen.
2. All stags were injected with intramuscular xylazine sufficient to achieve standing sedation.
3. All of the stags had a test tourniquet placed around both antlers by researcher (PF) or the farmer followed immediately by

4. Local anaesthetic, lignocaine 2% (nopaine 2%, Phoenix Pharm) in a ring block below the level of the tourniquet, applied by the farmer/veterinarian.
5. Both antlers were removed in the same order as local anaesthetic application.
6. Antlers were labelled with the identification of the deer and placed into a freezer for later grading and sampling.
7. At a later date the antlers were transported to a Takapau meat processor who band sawed off an approximately 100 g segment from the base of each antler.

Antler samples were placed into a chilly bag with ice packs and transported back to Massey University where they were sectioned in half. Half of each sample was sent to AssureQuality, Lower Hutt for lignocaine analysis. Samples were sent in three batches on the 01 November, 21 November and 6 December 2007.

Analysis was performed using Gas Chromatography and Mass Spectrometry (GCMS), using method XYL-03, Sedatives and Anaesthetics in antler and deer tissue by GCMS, issued May 2004 (AssureQuality). The limit of quantification for this method was 0.0047mg/kg and the coefficient of variation was 4.6% for 10 replicates of a single sample. Drug free control antler was obtained on a separate day from the Massey University deer research unit, using *NaturO*TM rings for analgesia.

During sample collection the following were recorded:

- Age of stag
- Sedation dose
- Tourniquet used
- Time of tourniquet application
- Time of lignocaine injection
- Volume of lignocaine injected
- Time of antler removal
- Haemorrhage during antler removal
- Head position and recumbency of stag
- Complications
- Weight and grade of antler

Between each session, modifications to the tourniquets were made by a contracted engineer, Palmerston North.

Tourniquet modifications

Table 4.7.1 (in the results section) gives a picture and description of the different tourniquet models that were used and also shows the identification numbers of the individual deer that each tourniquet was used on and the lignocaine result if antlers were tested for lignocaine residue.

Twenty tourniquets (or tourniquet application methods) were used in this project. Some of these were copies of the same design or method and were grouped for later statistical comparisons.

Model 1 in this experiment (Table 4.7.1) is the same as Model 9 in the previous chapter (4.6), except that the tension was increased as described below.

Increased tension

The model used during the 2006/2007 season delivered a tension of 6kg provided the jaws were not touching. As this resulted in 5/6 samples below the MPRL, the first modification made was to increase the tension applied using the same device. This was achieved by inserting a compression spring between the handles. This gave a tension force of 10kg provided the jaws remained open. (Models 1, 2, 3, 13, 14, 15, Table 4.7.1).

Inner tubing

In order to make the tourniquet more cost effective, and use readily available material, the spring clamp was fitted with thin (5mm) strips of either truck inner tubing or car inner tubing. (Models 2, 3, 11, 12, 13, 15). Inner tubing had elasticity allowing it to conform to the shape of the pedicle surface, was strong and did not deform when stretched tight multiple times. Inner tube can be cut to any desired thickness and length. Thinner inner tube for car tyres did not hold well in the clamp. Thicker inner tube from truck tyres held securely in the clamp at tensions up to 10 kg.

Cam cleat on disk

Lignocaine residues results from antlers that had been harvested while tourniquet models based on the spring clamp were in place were not consistently below the MPRL. Furthermore, A simpler and smaller fastening system was desired.

The cam cleat was fitted onto a small plastic disk with the rubber cord coming from one side. (Models 4, 5, 6, 7, 8, 11, 12, 16, 17). This fastening system was smaller and took less than 20 seconds to apply. It could be tightened after fitting by pulling the rubber up through the cam cleat. The model was easier to clean between stags compared to the spring clamp. The disadvantage of this model was that the tension could not be controlled or monitored. It was also slightly less secure than the spring clamp.

Spring in tube

In order to better control the tension in a smaller device, a system with a spring inside a double tube system was designed (Models 6, 9 and 10). None of these models were satisfactory due to the difficulty with placing the tourniquet, a large degree of haemorrhage in some cases and tourniquet slippage.

Double wrap

Lignocaine results from antlers harvested using the above modifications were not all available at the time that we needed to proceed with harvesting the rest of the antlers. Those that were provided showed inconsistent levels with many above the MPRL. Therefore it was decided to try wrapping the material twice tightly around each pedicle. This was done with the existing models (Models 11, 12, 13, 14, 15, 16, 17, 20).

Other models: Rubber bands and inner tubing and thin bungy cord

As interim results indicated that none of the modifications made were effective at reducing lignocaine residues, it was decided to test antlers from two commonly used methods for comparison. These included standard car inner tubing strips (Model 18) and size 77 rubber bands (Model 19). Car inner tubing was the same material used in the AgResearch study and resulted in all antlers complying with the MPL.

A thin (~ 2.5 mm) bungy material with greater elasticity was fitted to the existing devices. This was used during antler harvest before lignocaine results from previously harvested antlers were available. On reviewing previous results it was decided not to test these antlers due to the cost of testing and high probability of high lignocaine results as in previous tests.

Data analysis

Lignocaine residue data were tested for normality and were not normally distributed. Log 10 transformation resulted in an approximate normal distribution (Fig 4.7.1 (right)). Log10 has been used on the y-axis for most of the graphical displays for ease of interpretation.

Non parametric statistical comparisons included analysis of variance using Kruskal-Wallis test for comparison of medians, Mann-Whitney test for comparison of two samples and Pearson correlation test for correlation analysis. Differences were considered significant when $p < 0.10$.

For comparison of different tourniquets, these were grouped into six different “tourniquet types” (shown in Table 4.7.2).

Data were also examined for the effects of other variables on lignocaine residues.

A multiple variable logistic regression analysis was done using an online logistic regression analysis tool (Pezzullo and Sullivan, <http://statpages.org/logistic.html>) to test whether any of the independent variables had an effect on the binomial outcome of whether the residue level was greater than the MPRL.

Tourniquet wrapped twice vs once around each antler
Correlation between stag age and lignocaine residue

Table 4.7.1 Photos and descriptions of tourniquets used in the final testing stage in the attempted development of a standardised reliable tourniquet for prevention of lignocaine residues in harvested antler from mature stags. Lignocaine residues recorded from antlers harvested with each of the tourniquets in place are shown.

Model = Individual tourniquet or application method. Note that some tourniquets were similar in design, although independent units. Model 1 is based on Model 9 from Chapter 4.6 of this thesis.

Double = The cord was wrapped twice around each antler pedicle.

Same as = An independent unit that was the same design. Lignocaine concentrations: Green text <MRL, Black = 0.1 to 1.0mg/kg, Red >10x MRL

Model	Photo/description	Comments	Deer ID	Lig results mg/kg
1		 inner tube	153-01	0.21
			429-00	6.90
			429-00	1.20
			142-04	0.12
			142-04	0.76
2		Same as 1 inner tube	157-01	0.120
			157-01	0.017
			529-00	1.700
			529-00	0.190
			313-04	1.300
			313-04	0.049
3		Same as 1 inner tube	230-00	0.080
			230-00	0.089
			585-02	0.022
			585-02	0.036
4		5mm diameter neoprene	699-01	0.019
			699-01	0.072
			240-00	2.200
			240-00	0.810
5		5mm diameter neoprene	760-01	0.081
			760-01	0.014
			723-99	0.290
			723-99	0.650
6		DISCARDED lots of blood. Difficult to apply and tighten	413-00	0.13
			413-00	0.35

Table 4.7.1 continued

Model	Photo/description	Comments	Deer ID	Lig results mg/kg
7		same as 4	664-01	0.39
		5mm diameter neoprene	664-01	0.19
8		same as 4	453-00	2.90
		5mm diameter shock cord	453-00	0.59
9		Spring with no tube.	526-00	1.4
		Modification of 6	526-00	7.8
10		DISCARDED - Didn't work well, lots of bleeding and difficult to apply and tighten	874-02	results not taken
		Spring in a tube Modification of 6	874-02	
11	5 mm strip of rubber on cam cleat, similar to 8	Truck inner tube onto cam cleat on plastic disc.	102-03	0.23
		DOUBLE wrap	102-03	0.04
			571-04	0.17
			571-04	0.24
12	same as 11. thicker rubber (10 mm)	Truck inner tube onto cam cleat on plastic disc	290-02	0.520
		double wrap around each antler	290-02	0.061
			325-04	0.170
			325-04	0.014
13		Same as 1, wrapped double	186-01	0.076
		Plier clamp with truck inner tube double wrap CAME UNDONE	186-01	23.000
14		Same as 2, wrapped double	635-02	1.50
		Plier clamp with round section rubber double wrap. Same as 13	635-02	0.52
15		same as 3, wrapped double	140-02	1.0
			140-02	1.3

Table 4.7.1 continued

Model	Photo/description	Comments	Deer ID	Lig results mg/kg
16		Same as 7 wrapped double 5mm diameter neoprene	523-00	0.630
			523-00	0.042
			853-04	2.100
			853-04	0.960
			119-03	0.140
			119-03	1.100
17		Same as 8, wrapped double (same as 16) 5mm diameter shock cord	780-01	1.200
			780-01	
			565-04	0.015
			565-04	0.017
			820-01	0.031
			820-01	0.033
18	Inner tube only. Inner tubing from car tyres as used by the farmer on the property that the experiment was conducted on.		430-00	0.490
			430-00	0.052
			247-02	4.400
			247-02	2.700
			691-99	0.350
			691-99	2.400
			665-01	0.045
			665-01	0.054
19	Rubber bands size 77. Wrapped once around each pedicle and fastened with the cap of a hypodermic needle.		red 182	0.400
			red 182	0.380
			purple	0.680
			25	0.065
			purple	0.190
			25	0.340
			White 51	0.095
			White 51	0.060
			Green 54	0.130
			Green 54	0.230
20	Thin bungy double	Samples taken but no analysis done	Red 117	
			Red 117	

Table 4.7.2 Groupings of tourniquet type for statistical comparison of lignocaine residues. For each tourniquet type, the models that were used are shown along with the number of antlers tested for lignocaine for each tourniquet type and a description of the similar features of the models in each group.

Tourniquet type	Models (From table 4.7.1)	Number of antlers tested	Description
Original single	Models 1, 2 and 3	15	Based on Model 9 from Chapter 4.6 with a spring clamp and cam cleat to hold the tension. A single wrap of tourniquet material around each antler pedicle
Rubber on cam single	Models 4, 5, 7 and 8	12	Rubber cord used in a single wrap around each antler pedicle and fastened either on a cam cleat or a jam cleat on a disc.
Rubber doubled	Models 16 and 17	11	Rubber cord as used in “Rubber on cleat” but wrapped twice around each antler pedicle.
Truck inner-tube on cam doubled	11, 12, 13	8	Inner tubing from truck types which is thicker than inner tube from car tyres. Wrapped twice around each pedicle and fastened on a cam cleat.
Rubber band	19	10	A size 77 rubber band wrapped around each pedicle once and fastened with the cap of a hypodermic needle.
Standard inner tube	18	8	A strip of inner tubing from a car tyre wrapped at least twice around each pedicle and fastened by feeding a loop under the last turn around the pedicle.

Results

A full table of results is included in Appendix 4.7 (electronic version on CD).

Lignocaine residue results

Table 4.7.3 shows the overall distribution of lignocaine residues. Figure 4.7.1 shows the individual data points for lignocaine results included in the analyses on both a linear and log 10 scale with the MPRL indicated on the graphs. One value of 23mg/kg from a stag on which the tourniquet came undone between the time of lignocaine injection and antler removal and four values from stags on which a spring tension tourniquet was used were removed from analysis. This latter tool was unsuitable due to difficulty with placement and handling. These values were removed because of the confounding effect when assessing other variables in the data set.

Logistic Regression analysis

A regression analysis that included the variables: stag age, xylazine dose, tourniquet type, total volume of lignocaine, time from injecting lignocaine to velveting, haemorrhage score, and sitting during velveting was run to determine whether any of these variables had a significant effect on the binomial outcome “Residue above MPRL”. No variable in the model had a significant effect on the outcome ($p>0.1$).

Lignocaine result vs type of tourniquet

Descriptive statistics of lignocaine residues for each tourniquet type are summarised in Table 4.7.4. Figure 4.7.2 shows individual values and medians for each tourniquet type with lignocaine results plotted on a log10 scale. There was no statistically significant difference between any of the tourniquet types ($p>0.1$). The medians did not vary significantly as determined by 1-way ANOVA.

Effect of wrapping tourniquet material twice vs once around each pedicle.

Figure 4.7.3 shows the individual values and median values of lignocaine residues for velvet harvested from antlers that had the tourniquet wrapped either once or twice around each pedicle. Data are plotted on a log10 scale. There was no statistically significant difference in lignocaine results between antlers that had the tourniquet wrapped once or twice ($p=0.62$).

Effect of stag age

Lignocaine analyses results from antlers from stags of each age (3–7 years individually, ≥ 8 years grouped) are shown in Figure 4.7.4. There was no significant effect of stag age on lignocaine results. Regression curve fit showed no significant correlation for lignocaine vs stag age.

Table 4.7.3 Descriptive statistics for overall lignocaine residue values from antlers harvested after application of different tourniquet models

Results included	69
Minimum	0.014 mg/kg
Lower Quartile	0.0605 mg/kg
Mean	0.67 mg/kg
Median	0.21 mg/kg
Upper Quartile	0.79 mg/kg
Maximum	6.9 mg/kg
Proportion below 0.1mg/kg	36%
Number of results excluded	5
Excluded values	Outlier 23mg/kg ¹ , TQ type C ²
Kurtosis/skewness	Positive 12.9/3.4
Test for normality	Not normally distributed p<0.0001

1. Tourniquet became undone, result high and excluded from analyses
2. Type C tourniquet discarded due to technical difficulties, results excluded

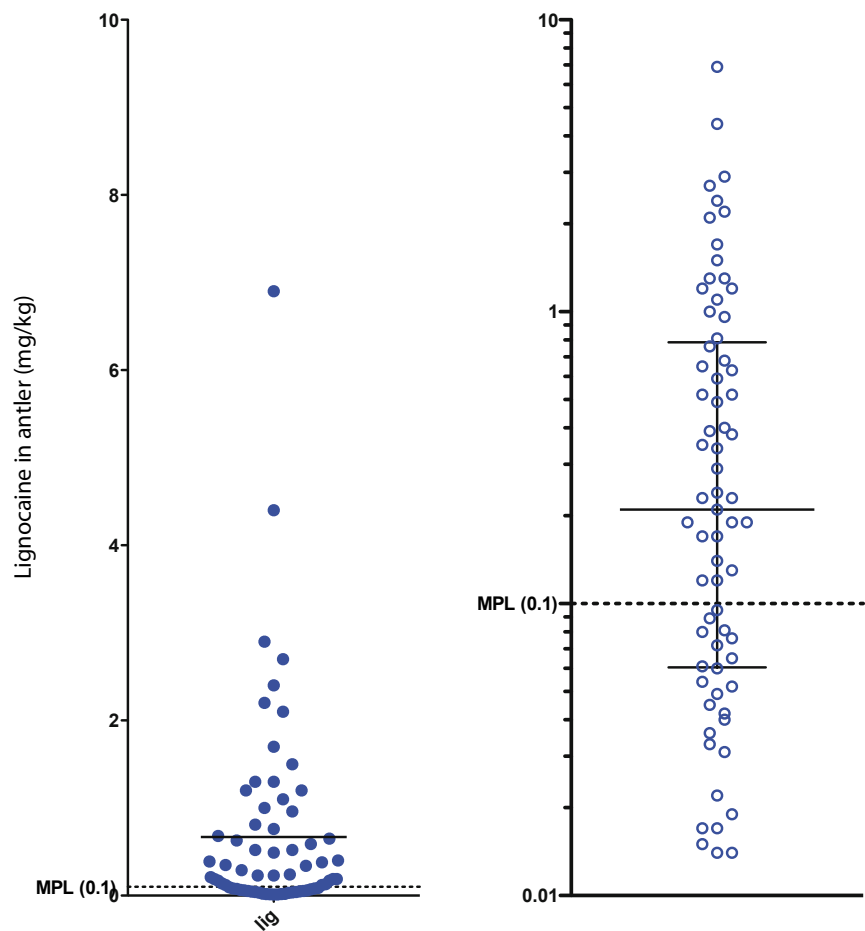


Figure 4.7.1 Lignocaine concentration (mg/kg of antler) from antlers of adult stags after velveting using different tourniquets for haemostasis. The range and distribution of all values (5 data points excluded due to tourniquet malfunction) are shown. The graph on the left shows linear values and illustrates the skewed spread of the data. The graph on the right shows data on a log10 scale with an approximately normal distribution.

Table 4.7.4 Descriptive statistics on lignocaine residue in velvet antler (mg/kg) harvested using six different types of tourniquet

Tourniquet group	Original single	Rubber on cam single	Rubber doubled	Truck inner on cam doubled	Rubber band	Standard inner tube
N	15	12	11	8	10	8
Minimum	0.0170	0.0140	0.0150	0.0140	0.0600	0.0450
25% Percentile	0.0490	0.0765	0.0310	0.0505	0.0800	0.0530
Median	0.1200	0.3400	0.1400	0.1700	0.2100	0.4200
75% Percentile	1.200	0.7300	1.100	0.2350	0.3900	2.550
Maximum	6.900	2.900	2.100	0.5200	0.6800	4.400
Mean	0.8529	0.6838	0.5698	0.1806	0.2570	1.311
Std. Deviation	1.761	0.9219	0.6950	0.1618	0.1957	1.648
Normality test (alpha=0.05)?	No	No	No	Yes	Yes	No
Proportion below 0.1mg/kg	40%	33%	45%	38%	30%	38%

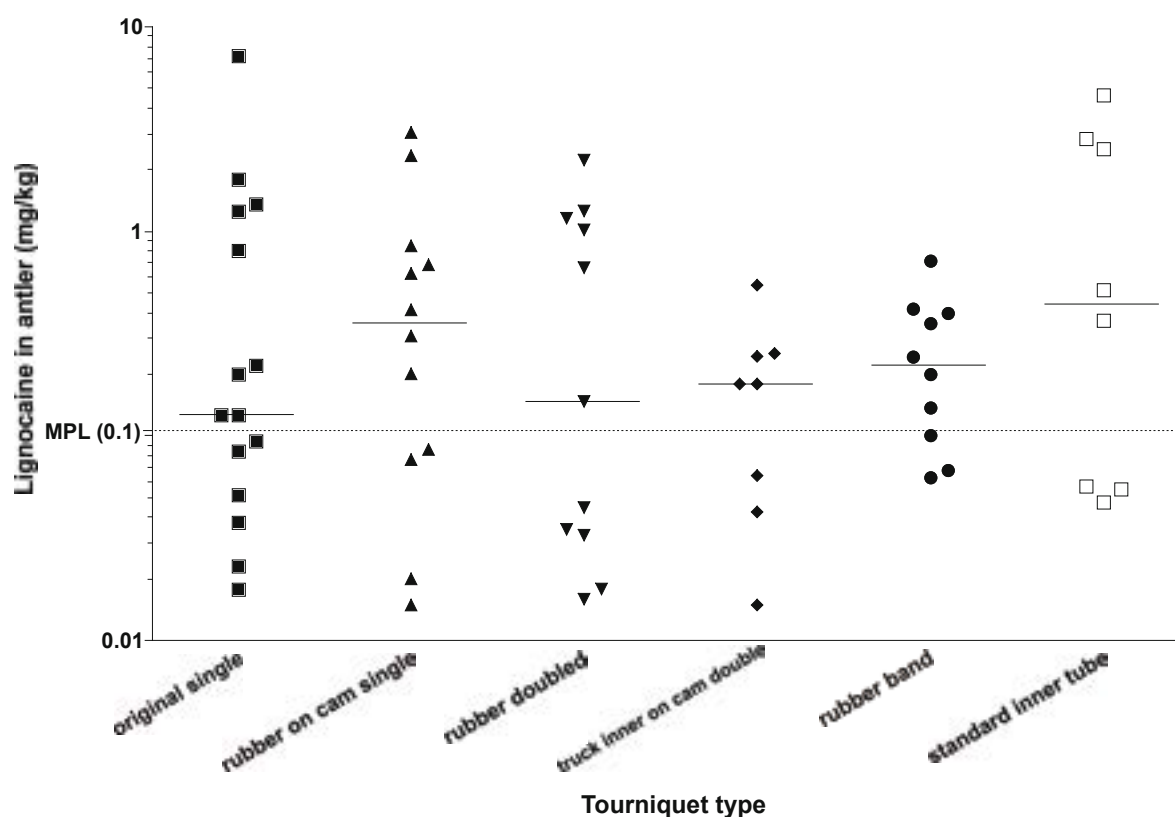


Figure 4.7.2 Lignocaine in antler harvested from mature stags for each different tourniquet type used for haemostasis. Individual values and median are shown for each tourniquet type. Dotted line represents the MPL of 0.1mg/kg. Note y-axis is log 10 scale

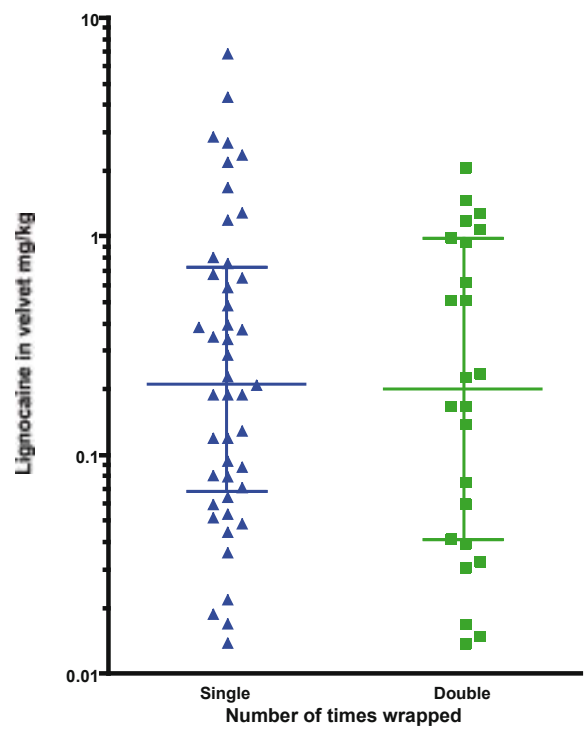


Figure 4.7.3 Lignocaine residue in velvet antlers of mature stags harvested when a tourniquet was applied either wrapped around each pedicle once (S) or twice (D), showing individual values and median.

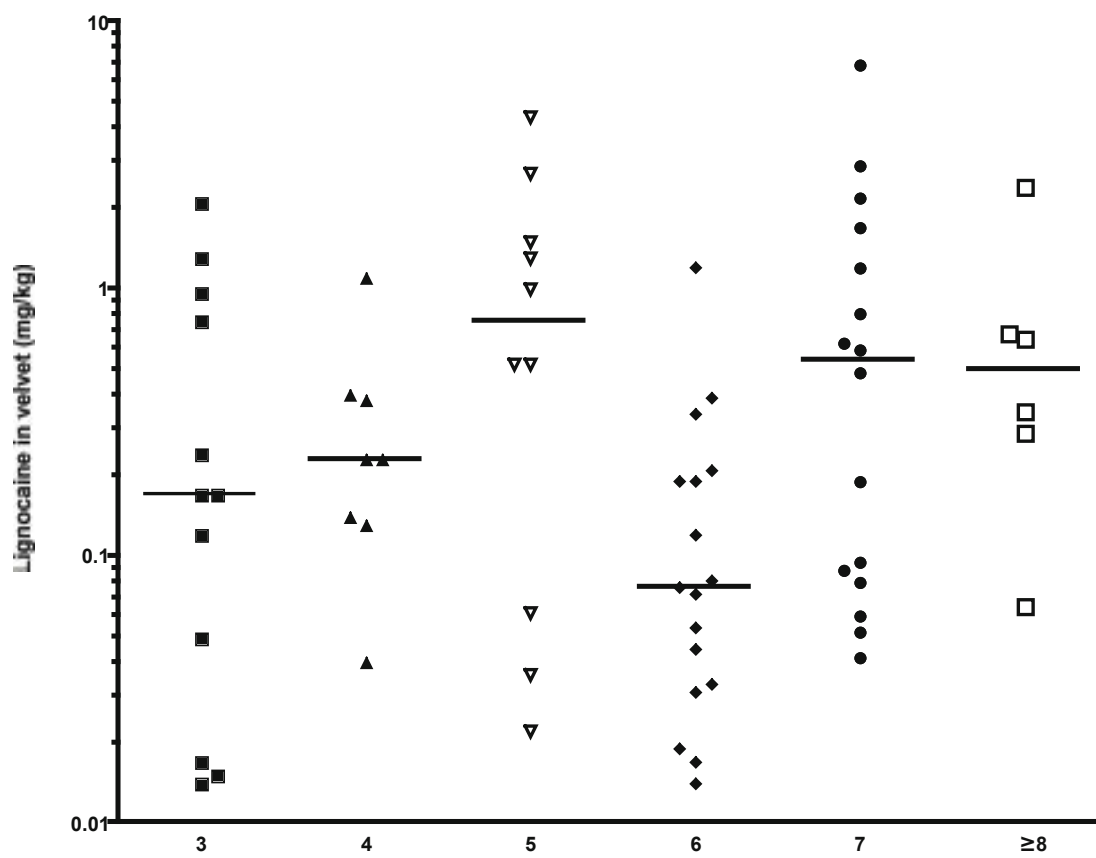


Figure 4.7.4 Lignocaine in velvet harvested from stags of different ages showing individual values and median. (Note y-axis is log10 scale)

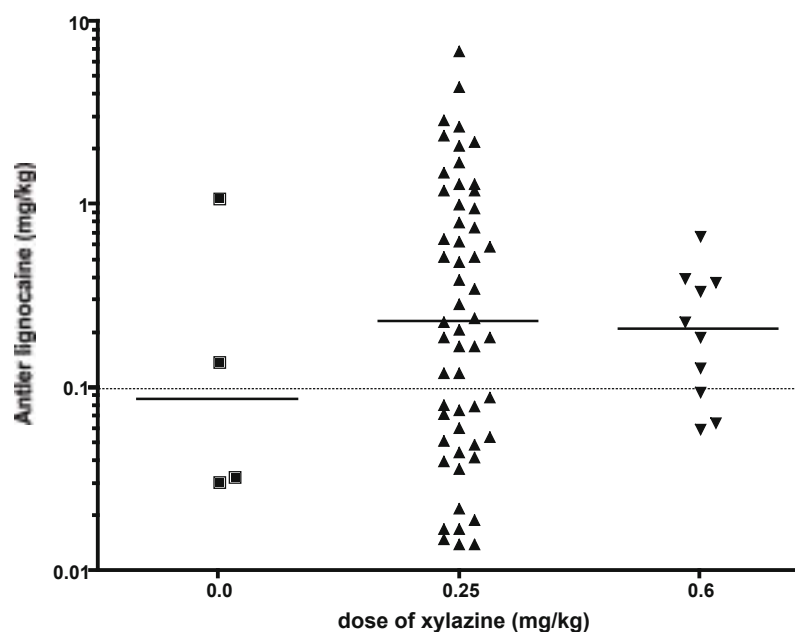


Figure 4.7.5 Antler lignocaine concentrations from adult stags that were velveted under 3 different dose rates of intramuscular xylazine: Nil, 0.25mg/kg and 0.6mg/kg. Dose rates are approximate. Data are shown on a log10 scale. Line is at the median for each group and the MPL of 0.1mg/kg is shown as a dotted line

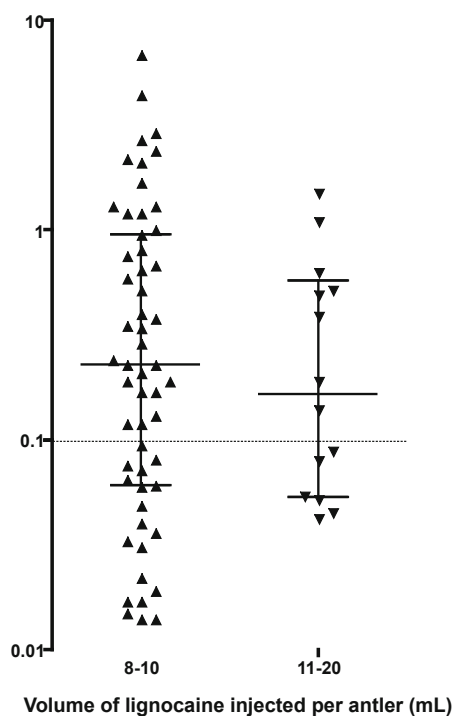


Figure 4.7.6 Lignocaine residue in velvet from stags injected with either 8 to 10 mL or 11 to 20 mL of local anaesthetic in a ring block around the base of the pedicle below the tourniquet

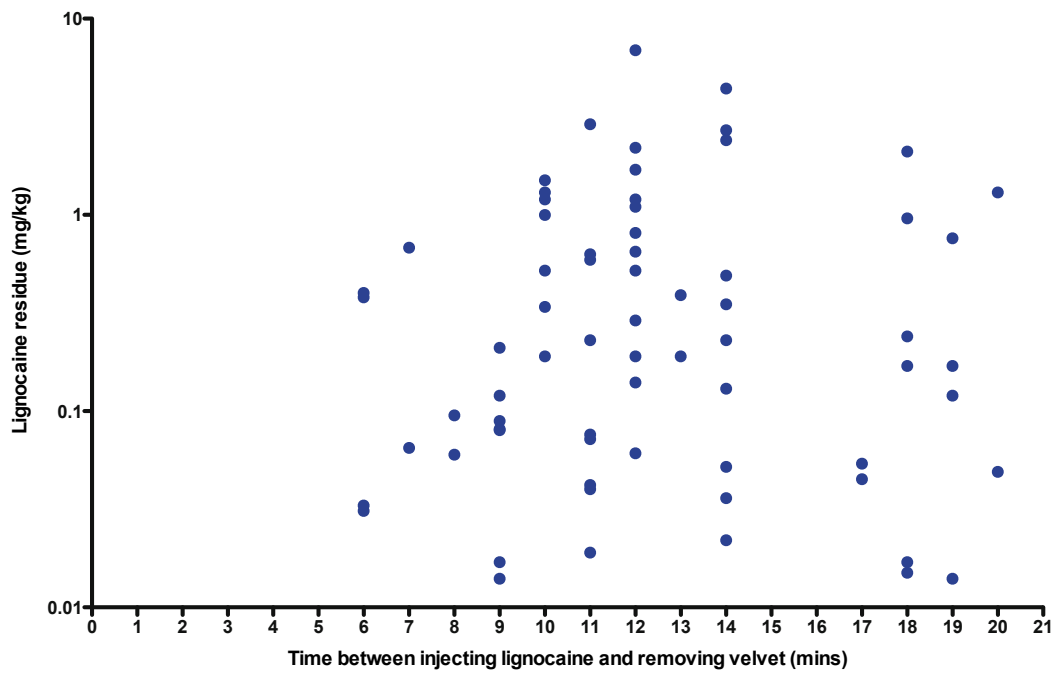


Figure 4.7.7 Scatterplot of the time waited between injecting local anaesthetic and velveting vs lignocaine residue measured in the same antler

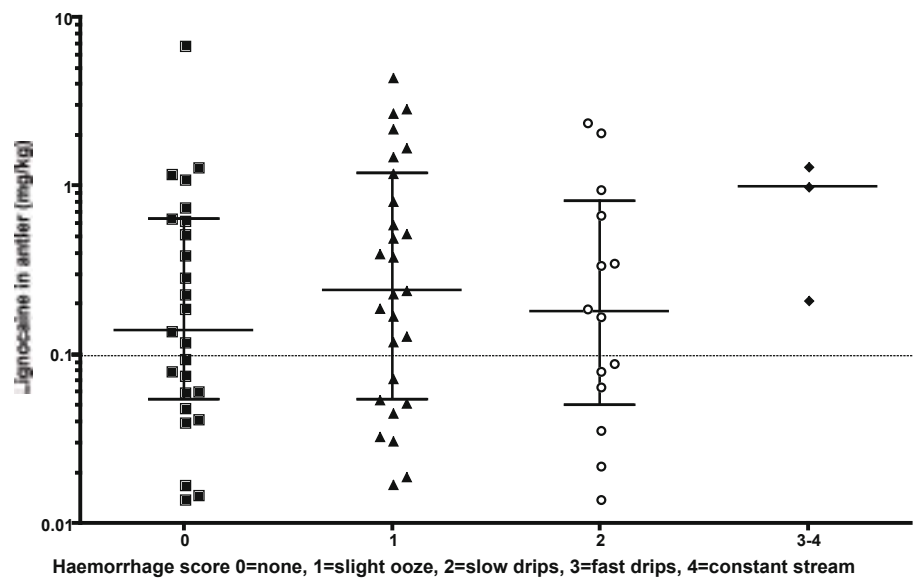


Figure 4.7.8 Lignocaine residues measured from velvet antlers from mature stags that were graded with different haemorrhage scores during antler cutting. Line is at the median and interquartile range. y-axis is log10 scale.

Effect of sedation

Figure 4.7.5 shows individual values and median of lignocaine residues in velvet antler of stags that were sedated with the following estimated dose rates of xylazine: None, 0.25mg/kg and 0.6mg/kg. There was no significant difference in the lignocaine residues in the velvet at different sedation doses. There was no difference between stags restrained in a crush or sedated during antler removal. The sample size of deer velveted in a crush (4 antlers) was insufficient to make valid comparisons.

Effect of recumbency

Fourteen antlers were removed while the stag was sitting down. There was no significant difference in antler lignocaine residues between deer that were standing or sitting during antler harvest.

Effect of injected volume of lignocaine

Volume of lignocaine used ranged from 8 to 20mL per antler. Pearson's test showed no significant correlation between injected volume and lignocaine residue in velvet antler. Antlers were grouped by injected volume (Fig 4.7.6) and there was no significant difference in the lignocaine residues in antlers that had been injected with a high (11-20 mL), or low (8-10 mL) volume ($p=0.35$).

Effect of time taken between injection of local and cutting antler

Time between injecting local anaesthetic and cutting the velvet ranged from 6 to 20 minutes (Fig 4.7.7). There was no correlation between the time between injecting and cutting and the amount of lignocaine in the velvet antler ($p=0.56$).

Effect of bleeding score

Bleeding score during antler cutting was graded from 0 to 5 using the following criteria:

0 = no bleeding

1 = slight ooze

2 = slow drips

3 = fast drips

4 = constant stream

5 = spurting

No antler was graded 5 during cutting and only three were graded 3 or 4. These were grouped for comparison. The highest residue level was in an antler that did not bleed at all during cutting. The three antlers graded 3 or 4 had lignocaine residue levels of 0.21, 1.1 and 1.3 mg/kg (Fig 4.7.8). There was no significant difference in lignocaine residue levels between groups based on bleeding score.

Discussion

Lignocaine residues were highly variable between animals and between antlers on the same animal. None of the tourniquets tested or any of the other independent variables that were measured had a significant effect on the amount of lignocaine in the antler within the parameters of this study. We failed to develop a tourniquet that reliably produced compliant lignocaine residues in velvet antler.

This is the first report of lignocaine residues in velvet antler harvested on commercial farms with different tourniquet types where other variables including dose of lignocaine and stag age have been recorded. Clear and Morris (2005) reported 50% of velvet antler from a commercial velvet pool had lignocaine residues above the MRL. There was no information concerning the use of tourniquets or method of analgesia and velvet removal from stags in that study. The range of lignocaine residue results from our study are similar to Clear and Morris (2005) although the rate of noncompliance was higher in our study.

If the hypothesis that lignocaine reaches the antler in the blood stream is correct then it would be expected that antlers that did not have complete haemostasis would have a higher proportion of lignocaine reaching the velvet. There was no statistically significant effect of the amount of bleeding seen during antler cutting and the amount of lignocaine in the antler. An outlier sample with a lignocaine residue of 23mg/kg was collected from an antler from which the tourniquet had come undone between injecting local anaesthetic and harvesting the velvet. Conversely, the antler with the second highest level of lignocaine had no bleeding at all during cutting. A similar proportion of antlers recorded as slow dripping when cut recorded lignocaine levels below 0.1mg/kg as antlers recorded as slight ooze or no bleeding. 43% vs 37% respectively. None of the antlers with a bleeding score of 3 or greater had compliant lignocaine levels, although the sample size was not large enough for this result to be meaningful. It is plausible that insufficient haemostasis may be related to higher lignocaine levels, particularly if partial haemostasis is achieved so that arterial blood is able to enter the antler but venous drainage is occluded resulting in pooling of blood in the antler as seen in the fluoroscopy study.

There was no effect of stag age on lignocaine residues. The minimum age of stags included in this study was three years. Previous studies (Bagonluri et al, 2005; AgResearch, unpublished; DINZ, unpublished) have used younger stags of two to four years of age. It was thought that lignocaine residue would be more likely in older stags that have larger diameter pedicles that are more difficult to access as there is a smaller space between the coronet of the antler and the frontal bone and the coronet tends to spread and overhang the pedicle, obscuring it from view and access for application of a tourniquet. Older stags may also have a greater number of irregularities on the pedicle surface within which the arteries supplying the antler could lie (see images in Chapter 4.6). As all the antlers sampled in the current study were from stags three years and over, it was not possible to determine whether mature stags have higher lignocaine residue than immature stags. Antlers harvested from yearling stags were kept, but these were not tested for lignocaine due to cost of analysis. Including tests from younger stags would have helped to determine whether residues are more likely to occur in older stags.

There was no correlation between lignocaine residue and the time waited between injecting lignocaine and cutting the antler. If lignocaine reaches the antler in the general circulation, it might be expected that the level of residue would increase with time. In the fluoroscopy study (Chapter 4.2, incomplete compression of the pedicle arteries resulted in a much longer retention time of contrast iodine in the antler vasculature. Alternatively, any lignocaine that does reach the antler may be removed from the tissue over time, so it may be possible to see a gradual decline in lignocaine residue over time. Neither of these effects were seen, indicating that either maximal levels are reached rapidly, redistribution from the antler is slow or the confounding variables in the model precluded identification in any trends over time. To further investigate this, it would be necessary to take repeated measurements from the same animal over time because the level of lignocaine in the antler cannot be predicted for a particular animal. As the antler can only be harvested once, this would not be possible.

One possible explanation for the noncompliant residue levels is surface contamination of the antler by the hands of the person injecting local anaesthetic. For a thirty gram sample to be contaminated so that it produced a result of 1mg/kg, would require 0.0015 mL of lignocaine on the surface of that section. It is feasible that this amount could be transferred to the antler from the hands of the operator or splash-back during injection at the base of the pedicle. We did not record antler handling during velvetting. This may provide useful information in the future. Alternatively, the hair could have been removed from the outside of the test antler sample before sending for processing.

Another possible explanation for the range of results is the potential absorption and redistribution of a relatively large volume of local anaesthetic from one injection site via a blood vessel system that is not occluded by the tourniquet. This may be through the central structure of the antler and pedicle itself. Alternatively small amounts of arterial blood may be able to move past the tourniquet transporting lignocaine without obvious bleeding observed during antler cutting. The model of the arterial supply to the antler (Chapter 4.6) showed that in some circumstances multiple arteries are stacked on top of each other about the periphery of the pedicle at the level of tourniquet placement.

The sample analysis was done under Good Laboratory Practice (GLP). Despite this there were some possible complications pertaining to the method and recovery results. Because the range of results was very large (10^3 difference), and it was not possible to predict the expected level in any one antler, the calibration curve needed to cover a wide range. Some of the results were outside the normal calibration range for this test. This may cause results to be relatively inaccurate at either end of the scale. Other published studies have had ranges over three orders of magnitude with very good correlation coefficients.

In the current study there was a small peak that co-eluted at the same time as lignocaine present in blank antler. Percent recovery methods were modified due to the presence of this peak. To compensate for this, isotopically labelled lignocaine was used. The size of the peak was small compared to the level of residue in test antlers and is unlikely to have affected the results.

Recovery ranged from 18 to 67% These recovery figures are lower and of greater variability than

other studies published in the literature. Recovery from plasma is usually greater than 90% (Coyle and Denson, 1986; Lorec et al, 1994). Recovery rates from antler tissue using gas chromatography have not been documented. Using HPLC, Bagoluri et al (2005) achieved recovery rates of 75 to 80% for the base and 68-73% for the tip and Cox et al (2005) reported a 90% recovery rate for lignocaine from pig microsomes, a solid tissue as opposed to serum, plasma or urine. The reason for the apparent low recovery in this project is unclear.

Calibration curves were made using blank samples harvested from one-year-old deer using *NaturO*TM rings. The recovery from spiker velvet may differ from that of adult stag velvet. This would affect all the results so that they were reported too high or too low. Using an internal standard would overcome this possibility.

As there was some concern regarding the accuracy of the results from the laboratory, three duplicate samples were sent for analysis using the same methodology, but not GLP. The results and comparisons with the original samples are tabulated below (Table 4.7.5). It should be noted that the duplicate samples were from the same antler at the same level above the pedicle. However, they represent opposite sides of a single slice and it is possible that the amount of lignocaine may be different across different regions of the slice. There was reasonable similarity between the sample results for sample A and B but sample C produced quite different results at the two testing occasions.

<i>Table 4.7.5 Results from duplicate samples sent toASURE- Quality laboratory on separate occasions</i>			
Sample	A	B	C
Original analysis	2.4	0.35	0.016
Repeat analysis	1.3	0.41	0.46

One option to help reduce lignocaine noncompliance may be to decrease the amount of lignocaine given at each injection site and in total dose overall. This could be achieved by injecting very small volumes over a large number of sites in combination with using 1% solution of lignocaine. If this method was to be adopted it would have to be at least as effective at providing analgesia as the current method for applying the lignocaine. We tested the use of 1% lignocaine as small injections over a large number of sites (data not shown) and found that a 1% solution was as effective as a 2% solution and that using a larger number of injection sites resulted in a smaller volume being used. In our experiment 5mL delivered over 20 sites was as effective at providing analgesia as 8mL over 4 sites. By using a 1% solution over multiple small injection sites, the total dose of lignocaine was reduced from 160mg to 50mg per antler. These antlers have not been tested for lignocaine residue due to the cost of analysis, however they have been kept in storage in case later testing becomes a viable option. Using a large number of injection sites is not practical because of the amount of time required to achieve this. Another option is to use modern injection gun technology in the form of a needleless multidose injection device that is able to rapidly inject small volumes of solution. Such tools are patented and thus available and may be an area worth investigating in the future.

Conclusions

Only approximately one third of antlers had lignocaine residue levels compliant with the current MRL of 0.1mg/kg. Noncompliant concentrations ranged up to 23mg/kg. Tourniquet type, bleeding, sedation, time from lignocaine administration to cutting, volume of local injected were not related to lignocaine in velvet antlers. Factors independent of tourniquet placement are likely to be responsible for antler residues and further investigation is required to understand these mechanisms.

Current tourniquet designs are not adequate to prevent lignocaine residues in antler.

Discussion on chapter 4

Summary of results from this chapter

Seven experiments are described in this Chapter.

4.1 Tourniquets of 1kg tension or greater reduced the number of fluorescent microspheres reaching the antler by at least 97.5% in all except one case but on no occasion did tourniquets completely prevent fluorescent microspheres entering the antler. On one occasion, microspheres were only reduced by 86.6% and this may be related to tourniquet placement and incomplete contact around the pedicle.

4.2 A tourniquet prevented contrast iodine entering the antler at tensions of 2kg or less provided the tourniquet was placed correctly.

4.3 A tourniquet prevented visible arterial spurting during velvet antler removal from non-sedated stags but did not prevent lignocaine residues in velvet antler.

4.4 A prototype tourniquet was developed with mechanical engineers and deer farmers.

4.5 One model with rubber cord was effective, accepted by farmers and tolerated by deer and prevented most lignocaine residues. Another was not effective at preventing lignocaine residues.

4.6 Antler casting and pedicle cross sections revealed elaborate arterial supply across the pedicle and irregularities of the skin, connective tissue and bone at the level of the tourniquet placement.

4.7 Field testing of a number of different tourniquet types and lignocaine residue testing found that tourniquets can be developed that are effective for haemostasis and easy to use but do not prevent noncompliant lignocaine residues.

The studies detailed in this Chapter have allowed us to gain further understanding about the nature of the blood supply to the antler and the occurrence of lignocaine residues in velvet. The results have alerted us to the fact that the vasculature pattern is more complex than originally thought and that prevention of lignocaine residues in antler may be more complicated than applying a tourniquet around the pedicle. There are many questions still to be investigated in order to develop the best solutions for overcoming noncompliant drug residue levels in antler.

Leakage of blood (Chapter 4.2, 4.3) and high lignocaine residues (4.3) were recorded at times during the experiments in this chapter and were thought, at that time, to be due to incomplete compression of the arteries around the pedicle due to the cumbersome nature of the tourniquet tensioning device. In Chapter 4.4, a tourniquet was developed that would provide a tensile force greater than 2 kg while ensuring a complete circle around each pedicle and fastening with a device that would not pull the tourniquet away from the surface at any point. The main aim of this experiment was to develop a tool that would be practical and acceptable to the farmer and the stag.

Ten antler samples were randomly selected during the experiment and tested for lignocaine residue. Of these samples, all antlers that had been harvested using the model with the rope material were noncompliant with the MRL and five out of six samples taken from antlers harvested using the rubber material were below the MRL. This adds further weight to the theory that leakage occurs because of inadequate compression around the entire pedicle. The rope material was easier to adjust and maintain a quantifiable tension on the clamp but resulted in higher lignocaine residue. The rubber material was more compressible and had a moderate degree of stretch. This resulted in more variable tension at the level of the clamp, but may have allowed the material to conform to irregularities around the pedicle surface. Another likely explanation for the observed differences between the types of material was a relatively small sample size from a population with a large variance and skewed distribution of lignocaine residue data. Later results support this theory. In Chapter 4.7, a larger number of antlers was tested (N=69) and gave a better indication of the true distribution of lignocaine residues in velvet.

Overall lignocaine results from all available sources are summarised in Table 4.8. The AgResearch and Massey data were provided courtesy of Velvet Antler Research New Zealand (VARNZ) and are confidential industry reports not published in the public literature. Permission to include these data has been granted by Deer Industry New Zealand (DINZ). Of the studies presented in Table 4.8, only Bagonluri et al (2005) and the research in this chapter included a well described method of quantification. The Massey results from Lab 2 included some zero results but none of the other samples reported antlers with zero lignocaine residues. The results from these studies appear to be similar except for AgResearch data which had lower lignocaine results overall with all antlers removed when a tourniquet was in place being less than the MRL of 0.1mg/kg.

Placement and dose of local anaesthetic do not explain the discrepancies between results of the studies outlined in Table 4.8 as the authors have given verbal confirmation that a high dose ring

Table 4.8 Results of lignocaine tests from different studies including those from this thesis

Source	Tourniquet?	N	mean	min	max
AgResearch (2005)	Inner tube	50	0.014	0.005	0.06
	None	22	0.272	0.057	0.82
Massey (2005) Lab 1	Inner tube	12	0.155	0.03	1.25
	None	11	0.30	0.07	1.46
Massey (2005) Lab 2 a	Inner tube	12	1.117	0	2.9
	None	12	0.91	0	3.5
Massey (2005) lab 2 b	Inner tube	12	1.1	0	5.1
	None	12	1.1	0	6
Clear and Morris (2005)	Not specified (some?)	50	50% were less than 0.1. Of those over 0.1 range was 0.12 to 18		
Bagonluri et al (2005)	None	42	0.48	0.14	1.37
Chapter 4.5	Rope model	4	values 0.41, 0.67, 1.3, 6.44		
	Rubber model	6	values 0.03, 0.04, 0.07, 0.07, 0.08, 0.51		
Chapter 4.7	Various	69	0.67	0.014	6.9

block using 2% lignocaine hydrochloride was used. Our study used a lower dose of lignocaine than these previous reports as it was conducted as normal farming practice by the farmer and vet that normally velvets those stags and not by the researcher. Volumes recorded were consistently lower than 1 mL/cm pedicle circumference.

The stags used in our investigations were all three years or older, whereas the AgResearch data were from younger stags. Older stags might be expected to have a greater number or size of arteries supplying the antlers as they are larger structures requiring a greater volume of blood for maintenance and growth. The surface may also be more irregular with blood vessels protected from the compression of the tourniquet. It is commonly observed (anecdotal observations) that the junction of the antler with the pedicle (coronet) of older stags grow closer to the skull each year, in some cases touching the surface of the skull making application of a tourniquet and local anaesthetic difficult. Final tourniquet testing (Chapter 4.7) did not support this as the residue levels were not correlated with stag age.

In order to move forward with research into lignocaine residues in velvet it will be necessary to develop a test for lignocaine that is cost effective and rapid. The variability and unpredictable nature of lignocaine residues, and the number of potential influences demonstrates that a large number of samples will be required to investigate any given treatments or interventions. The velveting season for first cut antler in mature stags is only four to six weeks long, therefore, rapid turn-around for results is paramount.

Methods for delivery of local anaesthetic

While much of the focus has been on developing tourniquet methods to prevent noncompliance with MRLs the solution may lie in the method of delivery and drugs used. If random high residues occur due to arterial spike from a single injection site or injection close to a non-occluded artery, then injecting a smaller volume, a lower concentration or using a needle-less injection device may reduce this risk.

Injection of a large number of small doses of lignocaine may be more effective at providing analgesia than injecting a small number of large volume doses (Chapter 2). This may be because a larger number of smaller volume doses assures that all nerves are exposed to local anaesthetic and thus the whole antler is better anaesthetised. This has the added benefit of administering a smaller dose at any one site and thus a smaller amount that may get into the antler from a poorly occluded region or from accidental artery spike. A multi-dose needle free injector (Mitragotri, 2006) may be a useful tool for achieving better distribution of lignocaine for analgesia with a reduced risk of lignocaine residues in velvet antler.

Drug concentration

One percent lignocaine hydrochloride is equally as effective as the same volume of two percent lignocaine hydrochloride for achieving analgesia of the antler in yearling deer. (Wilson et al, 1999) (Flint et al, unpublished). One percent lignocaine has not been tested in adult deer. Using a one

percent concentration can effectively halve the dose of active compound that is injected as the same total volume would be used. One percent and 0.5% are the commonly used concentrations in human medicine and higher concentrations are usually reserved for transdermal applications.

In a pilot study conducted at Massey University (Flint et al, unpublished) it was found that using 10 x 0.5mL doses of 1% lignocaine (50mg) had equal efficacy as 4 x 2mL sites using 2% lignocaine (160mg) for achieving analgesia in yearling stags. This presents an opportunity to reduce the total dose of lignocaine used and the potential for residue in antler.

Using a large number of injection sites is time consuming and unlikely to find compliance amongst farmers and vets in practice. Multi-dose needle free transdermal injection devices have been developed that would overcome this problem. Such a device may deliver a dose of 0.1 to 0.5mL per site and could be rapidly and easily moved around the pedicle to form a ring of anaesthetic. A needle free injector would also eliminate the risk of accidental arterial spike as no needles are inserted under the skin.

Alternative analgesic agents

Bupivacaine

Bupivacaine may produce less residue in velvet antler. The MRL for bupivacaine would be 0.1mg/kg according to schedule 1 New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards 2008. The standard concentration of bupivacaine is 0.5mg/mL. If the same volume is used for this formulation as for current practice using lignocaine, then the total injected dose of drug would be one quarter of the amount of lignocaine currently used. The metabolism and carcinogenic potential of bupivacaine are not well understood, but is likely to be similar to lignocaine as the structure is similar, with an aniline ring and two methyl groups at the 2 and 6 positions as for lignocaine. Bupivacaine has a higher pKa than lignocaine and a stronger protein binding affinity. This means that bupivacaine will take longer to take full effect but will have a longer duration of action than lignocaine. This has been shown to be the case in deer (Bartels et al, 2001) and the longer duration of action may be beneficial to the welfare of the stag.

Combinations of bupivacaine and lignocaine would make the onset of analgesia more rapid and the length of analgesia longer. As both have the same carcinogenic metabolite and according to Schedule 1 of the New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards 2008, lignocaine is the sum of lignocaine and 2,6 dimethylaniline and is expressed as 2,6 dimethylaniline, it is likely that residues for bupivacaine and lignocaine and 2,6 dimethylaniline would also be summated and expressed as 2,6 dimethylaniline.

Articaine

Another amide anaesthetic that looks promising is articaine (Vree and Gielen, 2005). This drug was first investigated for use in dentistry in 1973 and since 1983 has become the most popular drug for this purpose in Canada and several European countries. Articaine has a rapid onset of

action, being faster than or equal to lignocaine (Simon et al, 1997) and is at least as potent and effective as lignocaine (Johansen, 2004). Another attractive property is the fact that it is rapidly hydrolysed by serum esterases (Oertel et al, 1998; Vree et al, 1997) and breakdown in the antler may occur after antler harvest. It is not hydrolysed to PABA like other ester anaesthetics and is therefore likely to be safer than drugs such as chloroprocaine. Articaine is said to have good penetration through tissues including periosteum and bone. This makes it highly effective for regional anaesthesia in dentistry and may increase its efficacy for velvet analgesia compared to lignocaine. Articaine can be imported into New Zealand in small ampoules. The cost of production on a large scale for use in veterinary medicine would have to be investigated.

Other compounds

Alternative analgesic agents and devices are constantly being sought. Most of the commonly used and available compounds that provide rapid and complete loss of pain sensation can be metabolised to potentially carcinogenic products.

Ester anaesthetics have fallen out of popularity for human medicine since the invention of the amino-amides. The metabolite para-amino-benzoic acid causes allergic reaction in a relatively high proportion of people and there is very little indication for using amino-ester agents in preference to the superior amino-amide compounds. Chloroprocaine has been studied for velvet removal in deer, but the results have not been published (Wilson pers. comm).

The study into natural compounds for analgesia is, as yet, only in the early stages. Most naturally occurring compounds are too toxic or painful on administration or slow in onset in their raw form. Compounds that target nociceptors directly are of interest and have potential application for velvet removal. The deer industry should keep informed of developments in this area and consider early uptake of new methods, maintaining its status as industry leaders in adoption of new technologies for improving animal welfare.

Non-chemical analgesia has been studied. High pressure compression bands cause electroencephalogram, cardiovascular and behavioural changes indicative of pain. (Jonhson et al, 2005; Woodbury et al, 2005). Medium tension compression bands (Natur O rings) are currently considered acceptable for providing analgesia for one-year-old deer and NAWAC has recommended their ongoing use. Experiments at Lincoln University and Massey University have produced results for and against the analgesic and noxious effects of *NaturO*TM rings and is the subject of Chapter 3 of this thesis. Electroanalgesia is considered not effective and noxious during application (Woodbury et al, 2001).

If lignocaine and other amide local anaesthetics are considered to be a serious human health risk in the future then it will be essential for the farming industry to develop new and safe methods for rapid and complete analgesia. If this does not happen, the increasing concern for the welfare of production animals may preclude certain animal husbandry practices from being carried out.

Chapter 5

The effects of xylazine in red deer

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Introduction

The use and effects of xylazine are reviewed in Chapter 1.5. Xylazine is a sedative and analgesic drug that is widely used for restraint of deer during velvet antler removal. This drug has many properties that make it useful for sedation of stags including small volume that can be injected via the intramuscular route, rapid and smooth onset of sedation, relative safety to the animal and the operator and smooth and rapid reversal of sedation after intravenous injection of the alpha 2-adrenoceptor antagonist, yohimbine. Xylazine is not entirely free of adverse effects and the most important of these has been the sporadic death of stags up to 48 hours after injection of xylazine (Walker and Middleburg, 1988; Walker, 1989). The exact cause of the phenomenon is still unclear. Previous research relating to xylazine in deer and other ruminants is discussed in Chapter 1.5. Deaths may be due to direct effects of the drug on tissues, particularly pulmonary tissue that occurs in all deer and some deer may be more sensitive to these effects than others. Alternatively, a small population of stags may be susceptible to a hypersensitivity type reaction to xylazine or a combination of risk factors may lead to a fatal outcome in a small number of cases. The studies described here aimed to identify risk factors for post-xylazine stag death and develop a better understanding of the effects of xylazine in normal deer so that preventative measures can be investigated in the future.

The occurrence of stag deaths and potential risk factors are investigated by means of surveys of farmers and veterinarians. The direct effects of xylazine and detomidine on the oxygen carrying capacity of the blood and other haematologic changes are examined in a series of observations during student teaching classes and clinical situations.

5.1 Case report surveys of stag deaths post sedation with xylazine and xylazine combinations

Introduction

Post sedation deaths in stags are sporadic and unpredictable in their occurrence making it a difficult subject to research under experimental conditions. Information relating to the incidence, risk factors and pathophysiology of post xylazine stag death is sparse. Veterinarians and farmers who are at the scene when a stag death occurs have access to the animals and the information relating to that death which could not be anticipated in advance. This study aimed to collect information about stag deaths immediately after they had occurred in practice so that specific risk factors might be identified. Data were collected by providing a data collection book for veterinarians and farmers to complete in the field. Farmers who had deer die were offered a free sample analysis service using histopathology at Massey University. In addition, brief surveys and reports from previous studies were included in the analysis in the discussion in this section.

Note to reader: The layout of this section is unconventional. Data collected by the author are presented in the “Results” section but were insufficient for statistical analyses. Similar data that had been collected by VARNZ in years preceding this study were kindly provided and these have been combined (along with other data sources (Walker and Middleberg, 1988; Walker 1989)) with the data collected by the author in the present study to form a meta-analysis which is presented in the “Discussion” section. The “Materials and Methods” section relates only to the data collected in the current study.

Materials and Methods

Study design: Retrospective survey.

Case report data collection

A data collection booklet was prepared in hard copy and was also made available online. A copy of the data collection book is included in Appendix 5.1.1 The data collection booklet included question sheets and instructions as follows:

Form A: (white) General information about the farm

Please fill in only once

General information about the farm, facilities and practice

Contact details

Form B: (yellow) Velveting episode report

Please fill out one copy of this form (2 sides) for every velveting session during which a stag death occurs and at least 3 other episodes (When deaths do not occur) throughout the season. The more sessions we can get information on the better.

Velveting session is any occasion when any number of stags are velveted in a single shift: For instance on one single day if you velveted stags in the morning and another lot in the evening, it is considered as two episodes.

Form C: (pink) Stag death case details

Please fill out one of these forms for every stag that dies

Form D: (blue) Post Mortem form

Please call your veterinarian or take the carcass to the clinic for postmortem examination. If this will not be possible an on-farm postmortem instruction sheet is included at the back of the booklet.

It is critical that the postmortem be conducted as soon as possible after death and that the samples are submitted to the laboratory immediately.

Form E: (green) Laboratory submission form

Please ask your veterinarian to complete the laboratory submission form and send the samples to the address at the front of this booklet. If a veterinarian is not available you may fill in and send these yourself. Compensation will be paid for the cost of freight.

Completed forms were returned and the data were entered into spreadsheets for analysis. Post-mortem samples were examined for histopathology by John Munday at Massey University. Information was gathered from farms with and without stag deaths. On farms where deaths occurred, information from subsequent episodes when deaths did not occur was also gathered so that comparisons could be made between farms and between velveting occasions.

Notices to farmers and veterinarians were provided at the following places:

- Deer Farmers Association branch chairmen and subsequent branch newsletters
- Deer Industry News (official magazine of Deer Industry New Zealand and the NZDFA, Issue 17 December 2005)
- Notice in "Cervetec" the newsletter of the Deer Branch of the New Zealand Veterinary Association
- Email circulation to deer branch members of the NZVA

In the first season of data collection, insufficient data were returned so the project was continued for a second year. Data from both seasons was combined for analysis.

Brief incidence surveys

In addition to reports from individual cases, surveys of veterinarians and farmers were undertaken to gauge the extent of the issue compared to previous reports. These included:

- A brief survey of nine practicing veterinarians at the 2006 conference of the Deer Branch of the New Zealand Veterinary Association.
- A survey of 51 farmers from 3 separate field day meetings around the South Island.

These brief surveys asked the number of animals velveteed by the individual or the practice and the number of stag deaths that had occurred in the previous velvetting season and the total number that had been experienced by the individual.

At the end of the second year, there was still insufficient data for analysis. Data from this study was pooled with data collected by the NVSB during the 2001/2002 season and the 2002/2003 season and with data presented by Walker and Middleberg (1988) and Walker (1989) and forms the discussion of this chapter.

Results

Results – Stag death case report returns

Form completion and returns

A list of the number of stag death cases reported and form completion rates is provided in Table 5.1.1. Nineteen deaths were reported from 12 farms over the two year period. Twenty-seven velvetting episodes that did not include deaths were also reported. Of the 12 farms on which stag deaths occurred, 6 returned completed general farm information forms. Of the 19 stag deaths, 11 had stag death information forms completed (plus 3 that were partially completed (partial)), 7 had completed postmortem forms (plus 2 partially completed) and 6 had samples submitted for histopathology.

Two farms that did not have any deaths returned surveys. This was an insufficient number for comparative analysis between farms that did and did not experience stag deaths.

Location and herd size of case properties

Table 5.1.2 shows the location of the 12 properties that completed survey forms. Farms that experienced deaths were located from Auckland to Timaru. Four of the 10 farms that had stag deaths and one that did not have any deaths were from the Manawatu. One farm in Canterbury ran 4 different properties and all of these had at least one stag death. Herd size was not given for some of the properties and ranged from 6 to 260 per property for those that provided this information.

Velvetting environment

Yard and pen size, number of yards and pens, number of animals per pen and flooring and lighting conditions are shown in Table 5.1.3.

Table 5.1.1 Number of post-xylazine stag deaths reported and data collection forms returned from 12 farms that participated in a post-xylazine stag death survey. Also shown is the number of cases for which each farmer submitted samples for histopathology.

Partial: form was partially completed.

Farm	Number of deaths	General information (form A)	Episode (Form B)	Stag death case (Form C)	Post mortem (Form D)	Histopathology
1	1	yes	5	1	1	0
2	1	yes	5	1	1	1
3	3	no	0	3 partial	2 partial	3
4	3	partial	1	1	1	0
5	1	yes	3	0	0	0
6	6	no	0	5	1	1
7	1	yes	0	1	1	0
8	0	yes	3	0	0	0
9	1	yes	5	1	1	0
10	1	no	0	0	1	1
11	0	no	5	0	0	0
12	1	no	0	1	0	0
Total	19	6 +1 partial	27	11 +3 partial	7 +2 partial	6

Table 5.1.2 Location of farms, number of deaths and velvet herd size for farms that completed case report survey forms relating to post xylazine stag deaths.

Farm	Location	Number of deaths	Velvet herd size
1	Manawatu	1	6
2	Manawatu	1	14
3	Hawkes Bay	3	>100
4	Nelson	3	? >12
5	Manawatu	1	32
6	Canterbury (4 properties)		
6a	Timaru	1	not stated
6b	Spur road	1	260
6c	Richmond	1	160
6d	Mt Gerald	3	80
7	Timaru	1	23
8	Ashburton	0	62
9	Manawatu	1	23
10	Ashburton	1	Not stated
11	Manawatu	0	Not stated
12	Auckland	1	Not stated

Table 5.1.3 Yarding conditions on farms that returned post sedation stag death case report forms (at least one stag death occurred in these environments). Yard and pen size and number, flooring and lighting conditions and number of animals per yard or pen are shown.

Farm	No. hold- ing yards	Size hold- ing yards (m²)	Animals per yard	No. velvet pens	Size vel- vet pens (m²)	Animals per pen	Flooring	lighting
1	2	20	5 to 10	2	20	1	Bare con- crete	Natural
2	4	13.5-19	<5	3	13.5-19	4	Bare soil	Natural
3	NS*	NS	NS	NS	NS	4-8	NS	NS
4	1	20	<5	1	20	2	Bare soil	Natural
5	3	30-104	>10	3	4 to 6		Bare con- crete	Electric
6a	1	10-15		5	6	?	grating	NS
6b	8	5		30	3	2	bark/con- crete	NS
6c	3	7		3	7	2	dirt	NS
6d	3	5-15		5	4	2-3	dirt	NS
7	2	160		4	16	4	concrete	NS
8	5	15-20		5	4-5	3	concrete	NS
9	1	200		6	12-25	1-2	concrete	NS

*NS: Not stated

Farms had between one and four holding yards ranging in size from 13.5 to 200m². The number of animals in each holding yard before sorting into velveting pens ranged from less than 5 to more than 10 animals per yard. Velveting pens ranged in size from 4 m² to 25 m² and between 1 and 8 animals were held in each pen. Flooring was either concrete with no covering, concrete with wood shavings or bare soil. Most sheds were naturally lit, but one shed used electric lighting. Not all forms had all of the questions answered.

Time and Weather Conditions

The time of day and weather conditions during velveting episodes when deaths did and did not occur are shown in Table 5.1.4. Weather conditions included fine, sunny, cloudy, raining or showers and deaths occurred on days that were hot, fine, mild, showery or cloudy. Temperature was often not recorded, but ranged from 13 to 24°C on days when deaths were reported and from 12 to 24 °C on days that deaths were not reported. Wind speeds ranged from no wind to strong wind. Deaths occurred on days that were calm or had a light breeze but not when strong winds were recorded. Velveting occurred in the morning or the afternoon for episodes with and without deaths. Deaths occurred at various times of the day and were most often reported as “overnight”. Deaths occurred from less than 10 minutes to 48 hours after administration of xylazine.

Table 5.1.4 Weather conditions, temperature, wind conditions, velvetting time and time of death reported by farmers for each velvetting episode for which a form was returned. Multiple velvetting episodes were recorded on most farms. Episodes where deaths did and did not occur are shown on separate parts of the table.

Velvetting episodes when deaths did occur

Farm/ Episode	Temp °C	Weather	Wind	Start time	Finish time	Time of death
1/3	-*	Mild	-	-	-	10am
2/4	16	Showers	-	0730	0930	Overnight
3/1	-	Fine	-	-	-	<10mins post inj
3/2	-	Fine	-	-	-	10-15mins post inj
4/1	-	Fine hot	None	-	-	<1 hour post inj
6/1	-	-	-	-	-	9 hours post inj
6/2	15-20	Sunny	Calm	1700	1900	<12 hours, overnight
6/3	20	Sunny	Light breeze	0800	1600	<48 hours, overnight
6/4	15	Sunny	Calm	1700	2100	12 hours, overnight
6/5	24	Sunny	Light	1100	1600	<12 hours, overnight
7/1	14	Fine	Light	1000	1200	22 hours, 10am
9/6	13	Cloudy	Light breeze	1330	1600	24h, 4pm
12/1	18	Cloudy	Light breeze	1000	-	48hours. 3:30pm

Velvetting episodes when deaths did not occur

Farm/ Episode	Temp °C	Weather	Wind	Start time	Finish time
1/1	17	Cloudy	Light	1000	1100
1/2	17	Sunny	Light	1030	1200
1/4	18	Sunny	Light	1300	1430
1/5	?	Raining	Light	0815	0945
1/6	14	Cloudy	Light	0730	0930
2/1		-	-	0715	0900
2/2	12	Fine	Light	0700	0930
2/3		Fine warm	Windy	-	-
2/5		Sunny	-	0800	1200
5/1		Cloudy	Strong	1530	1630
5/2	16	Cloudy	Strong	1530	1635
5/3	15	Raining	Strong	1445	1625
5/4	18	Cloudy	Strong	1530	1645
8/1	15	Rainy	Calm	1500	1620
8/2	15	Cloudy	Calm	1000	1100
8/3	12	Cloudy	Calm	0730	0900
9/1	17	Sun/cloud	Calm	0945	1220
9/2	22	Sunny	Light breeze	1500	1555
9/3	13	Cloudy	Light	1045	-
9/4	16	Sunny	Calm	0930	1150
9/5	15	Rainy	Breezy	0845	1045
11/1	24	Sunny	Light Breeze	0930	1100
11/2	23	Sunny	Strong gusty	1500	1600
11/3	22	Cloudy	Light breeze	0730	0930
11/4	22	Cloudy	Light breeze	0730	0830
11/5	22	Drizzle	Light breeze	1335	1500

* - Not stated

Stress during mustering and yarding

Table 5.1.5 shows the answers to questions relating to stress during mustering and yarding and the number of broken antlers than occurred in deer during velvetting episodes when post sedation deaths did and did not occur. Not all farmers entered these details.

Deer were mustered either on foot or on motorbike with or without the assistance of a dog for episodes when deaths did and did not occur. Mustering difficulty was reported on two occasions when deaths occurred and on two occasions when deaths did not occur.

Stress in the yards or pens was reported in 4 out of 9 occasions when deaths did occur and in nine out of 25 occasions when deaths did not occur. The assessment of stress is subjective and some operators wrote more details than others relating to stressful behaviour.

Broken antlers were recorded during 5/9 episodes when deaths occurred and in 7/25 occasions when deaths did not occur. More than one stag with broken velvet was recorded in 3/9 episodes when deaths occurred but in none of the episodes when deaths did not occur.

Drugs Used

Not all farmers completed the drugs section of the survey forms. Where recorded, all drugs were within the expiry date and reported as appearing clear and normal in colour and consistency.

Sedation

Xylazine percent, stated general dose rate, route of administration and actual calculated dose based on volume used and estimate of stag weight are shown in Table 5.1.6 for stags for which data were provided.

All animals were chemically restrained with xylazine or xylazine plus ketamine. Some animals were given a top-up (repeat dose) of xylazine. None of the stags that died were given a top-up. When asked what dose rate was used, most operators stated 1mg/kg with one operator using 1.6mg/kg. When the actual dose given was calculated for stags that died, dose ranged from 0.3 to 1.6mg/kg. In all but one case, the actual dose given was less than the general dose stated in the velvetting episode form.

Reversal

Table 5.1.7 shows data relating to reversal of sedation using an alpha 2 antagonist. The drug name, dose rate and whether all or some of the stags were given reversal on each property are shown as well as whether stags that died were given reversal. On three properties, all stags were given reversal at most velvetting episodes. On three properties, some were given reversal and on five properties, it was not stated what proportion of animals received reversal. Reversal was adminis-

Table 5.1.5 Farmer reports of stress during mustering or yarding for each velvetting episode from farms that returned case report data collection forms relating to post sedation stag deaths. Episodes where deaths occurred are shown here and episodes when deaths not occur are shown on the next page.

Velvetting episodes when deaths did occur						
Farm/ Episode	Muster method	Muster difficulty	Stress in yard	Stress in velvet pen	Broken antler	Comments
2/4	foot	no	no	no	0	
4/1	foot	yes	some	some	0	slightly agitated, excited during yarding and penning
6/2	motor bike, dog	no	no	no	2	
6/3	motor bike, dog	no	some	none	2	panting and jumping over one another
6/4	motor bike	yes	yes	no	1	Difficulty mustering one animal that did not die. Panting, agitated and jumping in yards
6/5	motor bike	no	no	no	2	Semi drafted in paddock, 7 at a time
7/1	bike	-	yes	yes	0	A non-velveted stag was agitated and aggressive to the handler
9/6	-	-	no	some	1	2 animals jumping up wall
12/1	-	-	no	-	0	-

<i>Table 5.1.5 continued</i>					
Velveting episodes when deaths did not occur					
Farm/ Episode	Muster method	diffi- culty?	Stress in yard	stress in velvet pen	broken antler
1/1	motor bike	no	no	no	0
1/2	motor bike	no	no	no	0
1/4	motor bike	no	no	no	0
1/5	motor bike	no	no	no	1
1/6	motor bike	no	no	no	0
2/1	foot	no	no	no	0
2/2	foot	no	no	no	0
2/3	foot	no	no	no	0
2/5	foot	no	yes	yes	0
					Agitated/excited. Jumping over one another
5/1	motorbike and dog	no	no	no	0
5/2	motorbike and dog	no	yes	no	1
					Agitated/excited in yards
5/3	motorbike and dog	no	no	no	0
8/1	bike	no	none	yes	1
					jumping over one another
8/2	bike	no	none	none	0
8/3	bike	no	none	yes	1
					jumping over one another
9/1	motor bike	no	none	yes	1
					jumping
9/2	motor bike	no	yes	no	1
					1 3-year-old agitated and jumping
9/3	motor bike, dog	no	none	none	1
9/4	motor bike	no	none	none	0
9/5	motor bike	no	none	none	0
11/1	foot	yes	.	yes	0
					One stag was left on his own for the last hour and became unsettled, tried to jump over 7 foot jabbing race covered in heavy duty mesh
11/2	foot	no	none	none	0
					This time the stag had a 20 year old hind for company, he was calm all the time
11/3	foot	no	some	some	0
					panting, all had big heads grown out, probably wanted more pen room
11/4	foot	yes	Yes	none	0
					One a little upset at muster, brought in by himself. Panting and aggressive to handler
11/5	foot	no	no	yes	0
					Panting, humid weather. 3-4 spikers jumping over one another and a little upset

Table 5.1.6 Case report data relating to dose and route of administration of xylazine or xylazine combinations given to stags on farms that did and did not experience one or more stag deaths post sedation. Actual dose given to stags that died has also been calculated.

Farm	Product	Xylazine %	Dose stated	Route	Actual dose of xylazine- and route used in stag that died
1	Phoenix xylazine	10%	1mg/kg	IM neck	0.66mg/kg, neck
2	Xylazine 10%	10%	1mg/kg	IM neck	0.5mg/kg, rump
3	?	10%	?	?	0.3mg/kg, rump
	?	10%	?	?	0.3mg/kg, rump
4	Phoenix xylazine	5%	1.6mg/kg	IM rump	1.6mg/kg, rump
5	Phoenix xylazine	10%	1mg/kg	IM neck	?
6	Xylaze	10%	?	?	0.1, 0.5 and 0.3 mg/kg
7	Xylaket		0.9mL		0.2 mg/kg
8	Xylazine	5%	?	?	No deaths
9	Phoenix Xylazine	10%	?	?	0.6mg/kg
10	?	?	?	?	No deaths
11	?	?	?	?	No deaths
12	Phoenix xylazine and phoenix keta- mine	5%	2mL		0.56mg/kg

? = Not stated

Table 5.1.7 Data relating to the brand and dose rate of reversal (alpha 2 antagonist) drug administered to stags after sedation with xylazine and xylazine combinations. For those that answered this question, the table also shows whether all stags in the mob were given reversal and whether any that died were given reversal.

Farm	Reversal drug	Dose rate	All stags?	Stags that died?
1	Phoenix reversal	0.1mg/kg	all	not stated
2	Phoenix reversal injection	0.1mg/kg	all	yes
3	None		none	No
4	Phoenix reversal	4mL/250kg	some	yes
5	Phoenix reversal	0.1mg/kg	some/all	?
6	reversal injection	1 to 2 mL/stag	some	some
7	Tolazine	?	?	yes
8	No			
9	Phoenix reversal	1mL/100kg	?	yes
10	?	?	?	no deaths
11	?	?	?	no deaths
12	?	?	?	No

? = Not stated

tered intravenously, usually into the jugular vein and a dose rate of between 0.1 and 0.25mg/kg. Reversal was given between 20 and 30 minutes after administration of xylazine. At least five of the stags that died were given reversal. At least two stags that died were not given reversal.

Local Anaesthetic

Seven stags that died were given local anaesthetic by subcutaneous injection. One stag died before administration of local anaesthetic, and therefore velvet was removed post-humus without analgesia. On a different property, three stags that died on the same occasion were in hard antler and therefore no local analgesia was deemed necessary. The other that died was sedated for tuberculosis testing and there was no need for local anaesthetic. Dose rate of local anaesthetic ranged from 15 to 30 mls per animal. No adrenaline was used in local anaesthetic in any case.

Observations during sedation and antemortem

Observations that were recorded during sedation and before death are shown in Table 5.1.8. Five of the stags that died were seen to be having difficulty before death. Four of these were exhibiting respiratory distress and the other was bleeding from one antler eight hours after velvet removal. Two animals in acute respiratory distress less than 10 minutes after xylazine administration were gasping to breathe, but no airflow was detected. Another stag developed respiratory distress less than one hour after xylazine injection and was seen gurgling and panting and became recumbent. This animal was given reversal and "Depocillin" (a penicillin antibiotic) by the veterinarian. The fourth animal was observed wobbling and staggering one hour prior to death. This progressed to gasping for air, then sitting down and the animal finally became comatose and died.

Postmortem Findings

Gross pathological findings that were recorded by the veterinarian conducting the postmortem examination are shown in Table 5.1.8.

Ten stags were examined by postmortem. Reports were submitted for nine of these. Eight of the stags were in fresh condition at the time of postmortem. One was showing signs of postmortem degeneration. External abnormalities were noted in two animals. One of these (stag 2) had some evidence of bleeding from the antler and another (stag 10) had froth from the nostrils and mouth. Seven of the nine postmortem reports had stags with lung pathology. This included froth, sponginess, swelling, reddening and haemorrhage. Two of the stags had mild cardiac abnormalities and three were described as having reddened or haemorrhagic gastrointestinal tracts. One stag did not have any detectable abnormalities on postmortem.

Histopathology

Histopathological findings are shown in Table 5.1.9. Of the ten stags that had postmortem examinations, six had samples submitted for histopathology.

Table 5.1.8 Postmortem findings reported by the attending veterinarian or farmer in completing case report data collection forms relating the post sedation stag death. Only the significant pathological findings are listed.

Stag	Significant postmortem findings
1	Lungs: Pink, spongy and inflated with froth in the trachea. Thoracic cavity: Serosanguinous fluid.
2	Lungs: Swollen and slightly firm with patches of dark pink over 30% area Heart: Partially enlarged, a few red spots, no blood in lower chambers
3	Lungs: Spongy and inflated with some blood and patches of red
4	No report completed
5	External: Blood on thermometer from anus Lungs: Patches of red, lungs filled with blood Gastrointestinal: Abomasal folds swollen and haemorrhagic. Inner surfaces of SI haemorrhagic, blood in intestines
6	Lungs: Full of blood and fluid with patches of red on surface, lots of froth in trachea, no lungworm Heart: Blood tinged, straw coloured fluid in pericardial sac, red spots on surface, very little blood in chambers. Gastrointestinal: Abomasum: red-brown watery contents. Lining covered in 1-2mm haemorrhages, 1cm haemorrhage on exterior surface of omasum. Bloody jejunal contents, outer surfaces of jejunum dark red, 1-2mm red spots on outer caecum Bladder: Internal lining 1-2mm haemorrhages
7	No PM
8	No PM
9	No PM
10	External: White froth from mouth and nostrils Thoracic cavity: Increased straw coloured serum-like fluid Lungs: Pink/red marbelled, Spongy, froth and fluid filled, not inflated, froth in trachea, no lungworm Abomasum: Reddened, may be normal
11	No PM
12	No PM
13	No PM
14	No PM
15	No PM
16	Lungs: Large amount of fluid. Lungs not collapsed, interlobular oedema, patchy consolidation over 40%. Fluid flows when cut, free fluid 500mls
17	Post mortem change. Red spots of surface of heart
18	No abnormalities detected
19	No PM

Table 5.1.9 Histopathology findings from samples that were submitted to Massey University from stags that died after sedation during a case report study.

Stag	Significant Histological findings
1	No histopathology
2	Lung: Congestion moderate to severe with mild pulmonary oedema
3	Spleen: marked contraction. Almost no RBC present. Germinal centres prominent, neutrophils and eosinophils, indicating terminal severe stress. Kidneys: Interstitial nephritis, lymphocytic and neutrophilic, mild multifocal with proteinuria
4	Lungs: Bronchopneumonia, minimal, multifocal, acute, neutrophilic. small no.s neutrophils within and around bronchioles.
5	Lungs: congestion and haemorrhage, pleuritis, minimal, fibrosing and neutrophilic focal chronic Gastrointestinal: enteritis, mild, neutrophilic diffuse, subacute. Congestion and haemorrhage
6	No histopathology
7	No histopathology
8	No histopathology
9	No histopathology
10	Lungs: Pulmonary oedema, severe, acute marked with intralesional neutrophils Heart: myocarditis, focal chronic fibrosing, mild Adrenal glands: Congestion and haemorrhage
11	No histopathology
12	No histopathology
13	No histopathology
14	No histopathology
15	No histopathology
16	No histopathology
17	No histopathology
18	Kidneys: Interstitial nephritis, chronic active moderate lymphoplasmacytic with tubular necrosis and interstitial fibrosis Liver: 30% centrilobular hepatocyte necrosis. Acute centrilobular hepatonecrosis, moderate to severe.
19	No histopathology

Histopathology results generally correlated with the gross postmortem findings reported for those animals for which both were provided. The majority of pathological changes were again seen in the lungs and included pulmonary oedema, congestion and haemorrhage. Stag 5 had widespread congestion and haemorrhage in multiple organs, including the lungs, gastrointestinal tract and lymph nodes. Stag 3 had marked splenic contraction, with very few red blood cells seen in the spleen and germinal centres prominent. This is sometimes seen in terminal severe stress. This stag also had a mild interstitial nephritis, possibly due to leptospirosis, but no other significant findings. Stag 18, which did not have any detectable abnormalities on postmortem was found to have kidney and liver pathology but did not have any pulmonary pathology.

Results – Brief survey (Veterinarians)

Nine veterinarians completed a brief one-page questionnaire. Eight out of 9 had personal experience with post-sedation stag death and all had clients of their practice that had experienced post sedation stag death. The veterinarians estimated that they velveteed a combined total of 1750 stags per year and had collectively experienced 18 deaths in the previous season (2005/2006) (~1:100) and 94 deaths in their collective practicing lifetime.

Results – Brief survey (Farmers)

Fifty-one farmers completed a brief one-page questionnaire. These farmers estimated that they velvet a collective total of 18739 stags per year. 22/51 (43%) had personal experience of post sedation death in their stags. Nine stags were reported to have died in the previous velveting season (2005/2006) (~1:2000) and collectively a total of 50 stags were estimated to have died over the deer farming lifetime of these farmers. A space was also provided in the questionnaire for comments.

Comments included:

“Now always use a crush”,

“No longer sedate stags” from a farmer that has had a total of 6 stag deaths.

“One bottle of Rompum, I lost 3 stags”

“All through crush now, just local”, A farmer that has had 3 stag deaths.

“Pere David’s x Elk X red, 2yo stag OK after velveting but agitated 4 hours later, near dam, froth filled lungs, nasal passages, dense froth like foam, died in creek but head above water”

Discussion

There were insufficient data for statistical analyses from the present studies, therefore, data from a range of different sources including the data collection surveys in this study were collated and are discussed here. A list of the sources of information included in this discussion is shown in Table 5.1.10. Items 1 and 2 (Walker and Middelberg (1988) and Walker (1989)) are papers published in the deer branch of the NZVA conference proceedings detailing the clinical experience of the veterinarians in a practice in the Hawkes Bay. Items 3 and 4 are data that were provided by the National Velveting Standards Body (NVSB) from reports that had been submitted to that organisation during 2002/2003 and 2003/2004. Items 5 to 8 are data that were collected by the author of this thesis during the 2005/2006 and 2006/2007 velveting seasons.

Incidence

Estimates of the annual incidence of post-sedation death from different sources are shown in Table 5.1.11. The incidence of post xylazine stag death appears to vary from year to year. It is difficult to determine the incidence through a voluntary reporting system because very few cases are reported. It is likely that the incidence is grossly under estimated. Overall the range appears to be between 0.01 and 1.0% per year of stags that are sedated over the whole country. Some farms appear to have considerably higher and some lower rates of post xylazine death. Of the nine veterinarians who were surveyed at the 2006 deer branch of the NZVA conference, 8/9 had personal experience of with post-xylazine stag death and all had clients within their practices that had experienced deaths at some time. Of the veterinarians present approximately 94 stag deaths were recalled during their collective time in practice and the incidence reported by these veterinarians

Table 5.1.10 Sources of information used to investigate the occurrence and risk factors associated with death in stags after being given xylazine for sedation.

Item	Author	Year	Number of deaths	Source of data	Region
1	Walker and Middelberg (1988)	1988	30	Local practice clinical cases	Hawkes Bay
2	Walker (1989)	1989	2	Local practice clinical cases	Hawkes Bay
3	NVSB	2003	7	Reports to NVSB	National
4	NVSB	2004	6	Reports to NVSB	National
5	This study	2005	10	Case report data collection	National
6	This study	2006	9	Case report data collection	National
7	This study	2006	18 during past season 94 all years	Survey of 9 veterinarians at conference	National
8	This study	2006	9 during past season 50 all years	Survey of 51 farmers at field day meetings	South Island

was higher than all other sources of data.

When farmers were surveyed at a series of 3 field days in the South Island, the incidence was lower than for the veterinarians. Of the 51 farmers, 22 (43%) had personal experience with post xylazine deaths in their properties at some stage during their farming careers. 57% had never experienced a death. Approximately 50 stags had died on the 51 properties, with nine deaths occurring in the 2005/2006 season. This represents an incidence of 0.48 per 1000 stags per year, which is within the range reported by Walker and Middleburg (1988 and 1989). However, this is a significant underestimate of the real rate of adverse reaction because stags that were velveteed without any sedation (physical restraint only) were included in the total. It is estimated that 50% of stags are velveteed with local anaesthetic alone (Conway, 2005), thus the real risk of mortality after xylazine may be 1 per 1000.

Overall the incidence was approximately 0.1% per year. Because the deaths were unevenly distributed across years and properties this does not translate to a 1 in 1000 chance of death for an individual stag.

Spatial and Temporal Distribution

Table 5.1.12 shows the location, date and number of deaths for all farms for which this information was available from NVSB survey data and data collected for this study. Deaths were reported from both North and South Islands along the length the country. More individual reports were from the South Island than the North Island and this may reflect the fact that the majority of

Table 5.1.11 Number of stags velveteed, number of stag deaths reported and annual incidence of post sedation death from 4 surveys.

Source	Year	Total velveteed/ year	deaths in that year	Annual Incidence	Comments
Walker and Middleburg survey of 11 vet practi- ces in the Hawkes Bay	1986-1987	17256	30	0.17%	
Walker. Survey of 2 vet- erinary practices in the Hawkes Bay	1988-1889	15 000	2	0.013%	Deaths were reported from other practices not in the survey but no details were obtained
Survey of 9 veterinarians at NZVA conference	2005-2006	1750	18	1.0%	
Survey of 51 farmers at South Island field days	2005-2006	18700	9	0.048%	Includes non-sedated stags
Total		53 456	59	0.11%	

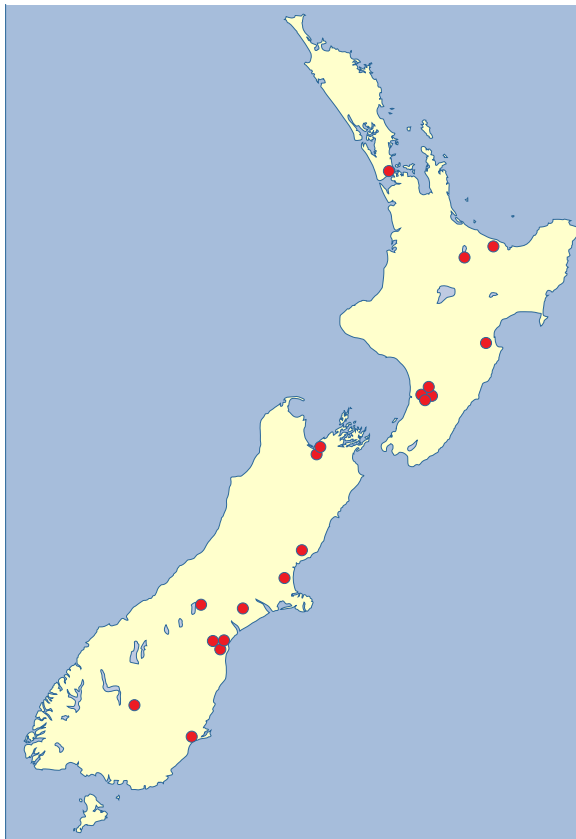


Figure 5.1.1 Location of farms that reported stag deaths following sedation to the NVSB or Massey University (this thesis) between 2002 and 2006

Table 5.1.12 Location of farms and number of deaths recorded after sedating stags with xylazine from farms that reported post-xylazine deaths to the NVSB or this thesis from 2002 to 2006.

Farm ID	Location	Date	Number of deaths
1	N Island	23/12/02	2
2	Otago	23/12/02	8
3	Dunedin	21/1/03	3
3	Dunedin	26/1/03	1
4	Nelson	28/2/03	2
4	Nelson	28/02/2003 died 01/02	3
5	Whakatane	3/03/2003 died Nov 02	1
6	Timaru	17/3/03	
7	Rotorua	7/4/03	1
8	Rangiora	17/11/03	1
9		14/11/03	2
10	Cheviot	14/12/03	3
11	Timaru	30/12/03	5
11	Timaru	31/12/03	2
11	Timaru	20/12/04	1
11	Spur road	30/11/05	1
11	Richmond	oct/Nov 05	1
11	Mt Gerald	16/12/05	1
11	Mt Gerald	5/12/05	2
12	Manawatu	29/10/04	1
13	Manawatu	17/12/04	1
14	Hawkes Bay?	24/12/04	2
14	Hawkes Bay?	17/1/05	1
15	Nelson	21/2/05	3
16	Manawatu		1
17	Timaru	3/11/05	1
18	Manawatu	1/10/06	1
19	Ashburton	18/11/05	1
20	Reporoa, Auckland	29/11/06	1

velvetting stags are in the South Island or that the survey populations were largely in the South Island. A cluster of reports were from the Manawatu region. This is most likely due to more active reporting by the veterinary practice in the region compared to other areas. Anecdotal reports from one North Island veterinarian of large numbers of stag deaths on a certain property along with the report from Walker and Middleberg (1988) of 30 stag deaths in the Hawkes Bay region during the 1987-1988 velvetting season suggests that there is no geographical preference for stag deaths *per se*. Although there may be certain factors occurring at a particular time that make stags in a particular area more susceptible to post sedation death. For example seasonal weather or pasture growth conditions.

Reports were made during the months of October to February inclusive. This is to be expected as this is the time of year that stags are sedated for velvetting and this only occurs during this time of the year. Sedation episodes that might occur at other times of the year include removal of hard antler during February to May or Tb testing of stags at any time of the year. One report of a stag death occurred following sedation for Tb testing and this occurred during the summer months. No reports of stag deaths were made following sedation for Tb testing at other times of the year. It is possible that deaths are more likely to occur during the summer or in association with velvetting or that velvetting itself is a major risk factor. The survey questionnaire format did not take account of deaths at other times. As the most common reason for sedation is velvet removal and there were few reports during velvetting, it is probable that there would be even fewer reports from sedations that are not associated with velvetting as these occur less frequently in practice.

Post sedation stag deaths appear to be clustered in time and on certain properties. Approximately one third of the episodes when deaths occurred (11/30) involved more than one animal dying at a single episode. Some people and properties appear to have a greater incidence of post sedation death than others. This is reflected in the farmer survey with approximately half of those surveyed reporting never having had a problem while others have ongoing problems to the extent that they have stopped using xylazine to sedate stags for velvetting and are now velvetting with manual restraint in a crush. The case reports to the NVCB and this project showed that 4 of the 20 properties experienced deaths on more than one occasion and one farmer that had deer on four different blocks of land experienced deaths on all four blocks. This study was not able to determine the specific risk factors for these farms and episodes.

Stress during mustering and handling in yards and pens

In the majority of cases, no stress or excessive excitement was identified when the stags were in the yards or velvetting pens. The reporting of animal stress is subjective. What one operator might consider to be stress may be considered normal behaviour by another operator. It may have been more useful to ask more objective questions relating to stress. In the first year of the survey, farmers were asked about the time taken to muster and time held in yards and pens. It was felt that the survey was too long and the initial data did not suggest any difference in these factors between episodes when deaths did and did not occur so the questions were not included in the second year. The number of broken antlers and other injuries may be a more objective measure of animal stress levels. This would be influenced by the size of the mob and the age of the deer. Young deer in

larger mobs are more likely to have broken velvet. Farm 6 had a relatively high number of broken antlers but was also velvetting large mobs at a time compared to other farms with between 10 and 70 stags velvetted at a single episode. Most of the other farms velvetted less than 10 stags at a single velvetting episode. Farmers were also asked the number of leg injuries and injuries leading to death or euthanasia. Only one episode reported two minor leg injuries. No post-sedation deaths occurred after that episode.

Breed

Of the stags that died, six were described as purebred Red deer, 5 were wapiti (or elk), 9 were Wapiti x Red, 2 were Danish White Elk and 27 did not state the breed. It appears that the proportion of Wapiti or crossbreds are over-represented relative to the number farmed in NZ. There is insufficient data to make firm conclusions about breed predisposition at this stage and the unreported breeds may have all been red deer. Two deaths have been verbally reported to the researcher in Père David X Red deer cross-bred stags. Thus all breeds included in the genus *Cervus* that are farmed in New Zealand appear susceptible. Deaths in Fallow deer (*Dama dama*) have not been reported. Multiple deaths have been reported in stags that are genetically related. There are not enough data to prove this in the current study. It is feasible that there is a genetic predisposition to post sedation death. If the mechanism of death is related to an alpha-2 agonist induced hypoxaemia and pulmonary oedema, it is possible that some stags are genetically predisposed to have a more severe reaction to the xylazine. One veterinarian reported reviving stags that went into acute respiratory distress by the immediate administration of reversal agent, antihistamine and adrenaline. These stags were tagged with a large orange ear tag marked X. On a subsequent occasion, the individual stags demonstrated the same response to xylazine injection and was again able to be resuscitated. (Hilson, pers comm). This report suggests some factor about the individual animal made it more likely to develop of fatal response to xylazine compared to others in the group.

Age

Table 5.1.13 shows the number of stags at each age that had case reports submitted to the NVSB or this study. There are more deaths reported in two-year-olds than in other ages. This may reflect that more two-year-olds are velvetted in total across the country or that they are more likely to be sedated for velvet removal. There were also a greater number of six year olds that died, although five of these were from one property. Older stags that have been previously velvetted and sedated without adverse effects have died at a later episode. This indicates that some change may have

Table 5.1.13 Number of stags of each age that were reported to the NVSB and Massey University as dying after being sedated between 2002 and 2006. NS=not stated

	Stag Age (years)										
	1	2	3	4	5	6	7	8	9	10	NS
Number	1	15	2	2	0	7	1	0	0	2	22

occurred within the animal or environment between these sedation episodes such that it has now reacted catastrophically. This is contradictory to the argument posed above that individual stags may be genetically predisposed to a fatal reaction to sedation. Death of only one yearling stag was reported. This may be because these animals are at a lesser risk of death, are less likely to be sedated for velveting or that spikers are perceived of lower value and therefore are less likely to be reported. At this stage, there are not enough data to support an age predisposition

Time from sedation to death

Table 5.1.14 shows the number of stags that died at various time intervals after xylazine administration. Most deaths (20) occurred between six and 48 hours after sedation. Three deaths were reported less than one hour after sedation. No deaths were reported between one and six hours. This suggests that there may be two mechanisms involved, an immediate possibly anaphylactic-type reaction and a delayed reaction. Although there are insufficient data to definitely show this to be the case and it may be that no deaths were observed between 1 and 6 hours because the stags tended not to be checked in this time period but rather later in the day or the following day. Most of the deaths that were more than 6 hours after sedation were reported to have occurred “overnight”, implying that the animal was found dead in the morning. The exact time of death and time after sedation would be difficult to tell in these cases. It is evident that a small number of stags experienced immediate respiratory distress and died within minutes of sedation but this is not the common presentation of the problem. There are not sufficient pathological data to say whether the mechanism is the same but more severe in these acute deaths as deaths that occurred between six and 48 hours after sedation. It is also unclear why stags would apparently recover and behave normally following reversal but then die after six hours. It would be expected that the effect of the xylazine would be wearing off by that stage and that stags should be fairly safe after 6 hours. That is clearly not the case in this disease. One possible explanation is that the xylazine triggers a locally mediated delayed inflammatory response in the lung parenchyma setting off a cascade of secondary mediators ultimately resulting in pulmonary oedema. To conclusively investigate the difference in susceptibility to acute and delayed reactions and the mechanism of the disease would require physiological studies on animals that were susceptible to dying. The difficulty with this approach is that these animals can rarely be determined in advance. As it has been observed that some farms have problems more than others, it may be possible to target these farms and perform

Table 5.1.14 Time after sedation that stags were reported as dying in case reports submitted to the NVSB and Massey University between 2002 and 2006

	Time after sedation						
	<10 mins	10-15 mins	15 min - 1 hour	1-6 hours	6-12 hours	12-24 hours	24-48 hours
Number of deaths	1	1	1	0	10	6	4
							NS
							17

more intensive monitoring of stags that are considered high risk.

Antemortem signs

Antemortem observations were available from only four cases as most animals were not seen prior to death, despite the Velvet Removal Programme requirement to frequently monitor deer after velveting. Three of these showed respiratory distress and one persistent bleeding from the antler. The pathology on the latter animal did not support blood loss as the primary cause of death. It is difficult to know whether stags that had moderate respiratory compromise that was not severe enough to cause collapse would be noticeable to the farmer. They would likely appear a bit slower and may have the head lowered but this may be a normal expectation for stags that had been sedated anyway. One animal that died was noted to be lying down but got up and attempted to flee on approach indicating that the animal was attempting to hide any signs of disease even though it must have been severely affected enough for it to later die. It is debatable whether more intensive monitoring post sedation would help to prevent future losses. Quiet stags that are well known by the farmer may show subtle differences in their behaviour that can be detected by the observant farmer. More fractious animals may not have any notable signs until close to death. If most deaths occur overnight those signs are likely to be missed.

The acute respiratory distress that was observed on one farm would be easily detected as these stags were recumbent and sedated in the pens at the time. They were observed to be gasping for breath but no air flow was detected. This would usually suggest an airway blockage, perhaps of the upper airway, but this seems unlikely in these cases. Alveolar collapse or emphysema or filling of the alveoli with fluid preventing air flow may present in a similar way. Given the postmortem and histological findings in these animals the latter seems to be the most likely explanation.

Gross Pathology

There were only a small number of postmortem reports provided and many were incomplete. These are summarised by organ system in Table 5.1.15. In most cases, there were no abnormalities reported except in the lungs. The most consistent and significant findings were pulmonary oedema with fluid and froth in the airways. Of the 13 postmortem examinations that were conducted, 2 specifically reported no abnormalities seen in the lungs. One of these was found to have renal and hepatic lesions on histopathology and the other did not have any samples submitted for histopathology. There were two cases with haemorrhagic enteritis which may be due to severe stress, but this is speculative. The significance of an enlarged heart and red spots on the surface and endocardial haemorrhage are not known.

Pulmonary oedema may be caused by left sided heart failure, fluid overload (e.g. due to kidney failure), pulmonary hypertension, traumatic lung injury, or a local inflammatory reaction with increased vascular permeability. Local inflammation of the lungs may be induced by an infectious condition such as bacterial, viral or verminous pneumonia. Some other non infectious agents and many drugs can also induce pulmonary disease including oedema. The mechanisms for drug-induced pulmonary oedema are not well understood but may occur by a direct effect of the agent on

Table 5.1.15 Gross pathological findings reported to the NVSB or Massey University from 2002 to 2006 from post-mortem examination of stags that died after administration of xylazine or xylazine combinations for sedation

External examination	• Evidence of scour • Blood on thermometer from anus • Slightly bloated, some rigor mortis. White froth from mouth and nostrils • All other reports found no abnormalities
Abdominal cavity	• No abnormalities seen in any animal
Thoracic cavity	• Serosanguinous fluid • Increased fluid, straw coloured, like serum
Heart	• Enlarged, red spots, no blood in lower chambers, chicken fat clot, petechial haemorrhages on all valves, more severe in ventricle • Red spots on surface • Enlarged, red spots on surface • Serosanguinous fluid in pericardial sac, red spots, very little blood in chambers • Endocardial haemorrhage
Lungs	• Pink, spongy, inflated, froth in trachea, no lungworm • Patches of dark pink (30%), swollen and firm, no lungworm • Patches of red, spongy inflated, some blood, no lungworm • Patches of red, lung filled with blood and fluid • Patches of red, areas of firm, froth in trachea, no lungworm • Pink/red marbled, spongy, froth and fluid filled, not inflated, froth in trachea, no lungworm • Large amount of fluid, lungs not collapsed, interlobular oedema, patchy consolidation (40%), fluid flows when cut, 500mL free fluid • Severe pulmonary oedema • Masses of very thick foam in airways
Intestines	• Areas of small intestine very haemorrhagic, red linings, blood in intestines • Jejunum very bloody contents, outer surface dark red, caecum contents blood tinged, many 1-2mm red spots externally, rumen lining peeling
Abomasum	• Abomasal folds very swollen and haemorrhagic • Red-brown watery contents, lining had 1-2mm haemorrhages. • Reddened, may be normal (veterinary comment)
Liver	• Bluish colour • Dark brown
Kidney/bladder	• Bladder internal lining 1-2mm haemorrhages

Table 5.1.16 Significant histopathological findings from samples that were submitted from case reports returned to the NVSB and Massey University from 2002 to 2006 from stags that died after being sedated with xylazine or xylazine combinations

Lungs	• Congestion, moderate-severe with mild pulmonary oedema • Bronchopneumonia, minimal, multifocal, acute, neutrophilic. Small number of neutrophils in and around the bronchioles • Congestion and haemorrhage, pleuritis: minimal, fibrosing and neutrophilic focal, chronic. • Pulmonary oedema, severe, acute, marked with intralesional neutrophils
Heart	• Myocarditis, focal, chronic, fibrosing, mild subacute
Intestines	• Congestion and haemorrhage
Abomasum	• Congestion and haemorrhage
Spleen	• Marked contraction, almost no RBC, germinal centres prominent, neutrophils and eosinophils, indicating terminal severe stress
Kidney	• Interstitial nephritis, lymphocytic and neutrophilic, mild, multifocal with proteinuria. • Moderate lymphoplasmacytic with tubular necrosis and interstitial fibrosis
Liver	• 30% centrilobular hepatocyte necrosis, acute centrilobular hepatonecrosis, moderate to severe
Adrenal	• Congestion and haemorrhage

Table 5.1.17 Diagnoses made based on clinical observations, postmortem examination or histopathology for the cause of death in stags that died after sedation with xylazine and had case reports submitted to the NVSB or Massey University

Stag	Antemortem	Postmortem	Histopathology
1		Severe pulmonary oedema	Severe pulmonary oedema due to anaphylactic reaction
2		Classic allergic reaction	
3		Classic allergic reaction	
4	Delayed hypersensitivity	Delayed hypersensitivity	
5			Acute pulmonary congestion, cause unknown, not consistent with blood loss
6	Hypersensitivity	Very acute allergic reaction	Stress leading to hypovolaemic shock
7			Not determined, mild lesions only
8	Anaphylaxis or MCF	Very acute MCF or anaphylaxis	Massive vasodilation
9	Stress	Stress	
10	Delayed hypersensitivity		
11			Chronic renal disease may have predisposed the stag to acute hepatocellular necrosis with sedation.

cells that release mediators such as histamine or interleukins that are chemotactic for neutrophils or granulocytes (Miller et al, 1992). TNF alpha has been identified as one factor causing increased permeability of pulmonary vasculature (Stephens et al, 1988).

Histopathology

Table 5.1.16 lists the histopathological findings from stags that died after administration of xylazine for each of the organs systems. The most prominent findings were in the lungs with three of the cases showing a pulmonary neutrophilia indicating an inflammatory reaction. There was also some evidence of pulmonary infection in some cases. Other organs were usually mildly affected and there was some evidence of stress-related disease.

Histopathological findings were inconsistent between studies. Walker and Middleberg (1988) found abnormally large numbers of eosinophils in the lungs but this was not a finding in the samples submitted to Massey University which tended to show increased numbers of neutrophils in the lungs. More research in this area is warranted to determine the cellular changes and degree of oedema that occurs in normal sedated animals. There are not sufficient data to tell whether the stags had underlying pathology before sedation that caused them to have a fatal reaction to the sedation or whether they had normal healthy lungs prior to sedation and the xylazine caused all of the changes observed. In some cases the histopathology suggests a more chronic underlying condition. Lungworm is a common parasite of deer and generally induces an eosinophilia in the lungs. There was no evidence of lungworm in any of the post mortem reports submitted here. Lungworm may have been present in the stags that were studied by Walker and Middleburg (1988). Although the pathologist in that case reported that the level of eosinophilia was much greater than what is ever seen with lungworm infection.

There were not increased numbers of pulmonary intracellular macrophages seen by the pathologist nor was there any report of changes or degeneration of macrophages. Damage to pulmonary intravascular macrophages was a significant finding in sheep that were studied by Celly et al (1999). It is possible that the effects of alpha 2 agonists in sheep are similar to those in deer. If that was the case we would have expected to see some changes in the local macrophages.

Diagnoses

Diagnoses, where given, were made either on clinical presentation, post-mortem inspection or histopathology, and are presented in Table 5.1.17.

Diagnoses have been made in ten cases. All others did not state a diagnosis or the results were inconclusive. There are a number of reasons, other than a reaction to the drug, that stags may die after sedation with xylazine, and diagnoses reported in these cases included delayed or acute hypersensitivity, possible stress related conditions and possible Malignant Catarrhal Fever. The antemortem, postmortem and histopathological diagnoses did not always concur.

Time and weather conditions

Temperature when deaths occurred ranged from -20 to +30°C with most recorded between 15 and 24°C (Note: the report of -20°C may be an error since it is unlikely that this temperature would be experienced in New Zealand during the months that stag deaths were recorded). Temperatures ranged from 12 to 24 °C in situations recorded when deaths did not occur. Weather was reported as either sunny, fine, warm, showers, average, mild, overcast, still or light breeze when deaths occurred and sunny, fine, warm, rainy, cloudy or drizzly when deaths did not occur. Sedations occurred at times ranging from 7:30am to 9pm. While some farmers believe that an adverse weather event (e.g. South westerly change overnight) has been important, a number of reports have stated that the weather was fine overnight when the stag died. There is no suggestive evidence that the weather conditions or time of the day that sedation occurred had any effect on the likelihood of a stag dying.

Drugs

Table 1.1.18 list the different products that were used, the number of episodes during which each was used, the number of deaths that occurred and an estimate of the number of stag sedated

Table 5.1.18 Number of occasions (episodes) that different formulations containing xylazine were used to sedate deer on farms that submitted case report survey forms to the NVSB or Massey University between 2002 and 2006. Also shown is the number of deaths reported in total and the number of deer that were sedated with each formulation where that information was provided.

Drug Name	Episodes when deaths occurred			Episodes when deaths did not occur		
	Episodes	Deaths	Number sedated	Episodes	Deaths	Number sedated
Parnell Fentazin	2	3	?	0	0	0
Parnell Xylase 10%	5	6	130 + ?	0	0	0
Parnell Xylase Forte	1	1	?	0	0	0
Lloyd's AnaSed 10%	2	7	?	0	0	0
Phoenix xylazine 10%	5	8	79 + ?	10	0	30
Phoenix xylazine 5%	1	3	12	0	0	0
Phoenix xylazine unspec conc	3	13	?	0	0	0
Phoenix xylaket	2	2	4 + ?	0	0	0
Phoenix xylazine + phoenix ketamine	1	1	6	0	0	0
Xylazine injection 10%	3	3	46 + ?	7	0	33
Xylazine injection 5%	1	2	?	4	0	38 + ?
unspecified	3	3	?	5	0	25 + ?

? = unknown

during these episodes (when stated). Also in the table is the number of episodes reported when using each drug when no deaths occurred. All drugs were within the expiry date and reported as appearing clear and normal in colour and consistency.

All animals that died were chemically restrained with xylazine or drug combinations that included xylazine. Some animals were given a top-up (repeat dose) of xylazine, although none of the stags that died were given a top-up. No other alpha-2 agonist drugs other than xylazine were used although these are readily available and commonly used in horses. This is probably because there are xylazine containing formulations that are registered for use in deer whereas the products containing detomidine, medetomidine and romifidine are not. The current study did not investigate post velveting deaths in stags that were not sedated with xylazine combinations. Deaths in stags that had large hard velvet antlers removed with the stag restrained in a crush and no sedatives given have been anecdotally reported by a veterinarian in Canterbury (R Dunbar, pers. comm.). There was no postmortem or clinical pathology provided for these animals and it can not be assumed that the same problem is responsible as the post sedation death that is the subject of the current investigation.

There are 16 products currently registered with the ACVM (Chapter 1.5, Table 1.5.1) that contain xylazine and all of these are labelled for use in deer. The brands of sedatives used and the number of episodes when deaths occurred and episodes when no deaths occurred are shown in Table 5.1.19. Unfortunately there were missing data in the returned forms and the brand of drug used or concentration was not always specified. Most of the reports are from episodes when stag deaths occurred because of the nature of the data collection method, particularly that acquired by the NVSB which was only from episodes when stag deaths occurred and did not request information from other velvetting episodes without deaths. Consequently there were several products for which there were no episodes without deaths recorded. During episodes when deaths did occur, there were always many stags that survived the sedation with the given product. The results between episodes when deaths occurred and when they did not occur can not be compared directly. What is evident is that deaths have occurred when many different xylazine containing formulations from different manufacturers have been used. There is no evidence from this data set that any one product is a higher risk than the others. The brand, formulation and concentration of sedative appear to be unrelated to stag deaths. It appears that the reaction is occurring either to the xylazine itself or to some compound common in all formulations or to the act of sedation irrespective of agent. It has been commented by some individuals that they have lost a number of animals using drug from a particular bottle or batch. It is not possible to determine if this is due to the drug or other predisposing factors that are putting animals in the same environment at risk over the period that the product was being used.

Dose Rate

Overdosing does not appear to be a cause of post-xylazine death. Doses of xylazine up to 4mg/kg have been safely used in red deer without adverse effects (Janicki et al, 2006) and the product information sheet for "Xylaze"(Bomac laboratories ltd.) states recommended dose rates for immobilisation of up to 3mg/kg xylazine along with up to 3mg/kg of ketamine for red deer. The dose rates reported in the current study ranged from 0.1mg/kg to 1.6mg/kg intramuscular xylazine. While 1.6mg/kg would be considered a high dose rate capable of inducing profound sedation it is

not outside of the safety limits. Stag deaths occurred at low dose rates of 0.1mg/kg. This dose rate would cause light sedation with the animal remaining standing. Figure 5.1.2 shows the dose rates reported for stags that died and the dose rates reported for episodes when stags did not die. Note that the dose rate for stags that died was calculated based on the volume and concentration of the drug used and the bodyweight of the stag as indicated in the completed data collection forms. The dose rate used in episodes when stags did not die was based on the estimated dose rate across all stags. As individual weights of these stags were not provided it was not possible to accurately calculate dose rates. In some cases, when a dose rate for the episode was given and a stag death also occurred at that episode, the two values were different with the calculated dose in the stag that died usually being lower than the stated dose rate used in all stags at that episode. This indicates that farmers and veterinarians tend to give a lower actual dose of xylazine than anticipated.

Reversal

The sedative, muscle relaxant and analgesic effects of xylazine can be reversed by giving an alpha-2 antagonist such as yohimbine. Of the stags that were reported as dying following sedation, 10 were given reversal at a dose rate of between 0.025 and 0.16mg/kg, 3 were not

given any reversal and 16 did not state whether reversal was given or not. Reversal was administered intravenously, usually into the jugular vein between 20 and 30 minutes after administration of xylazine. Across all of the sedation episodes, reversal was given more often than not. The dose

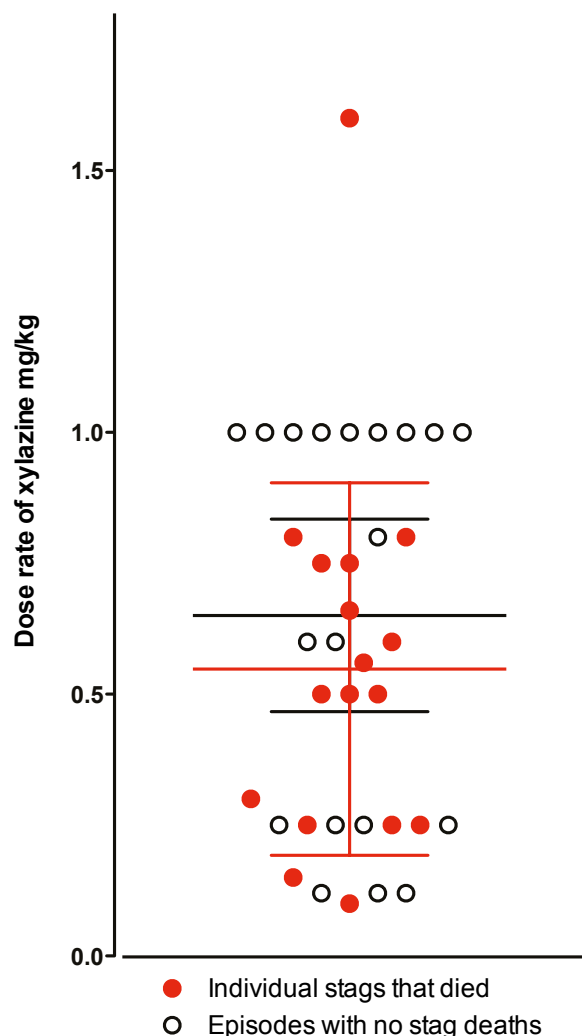


Figure 5.1.2 Dose rate (mg/kg bodyweight) of xylazine administered to stags that did and did not die after sedation as reported in case report data collection forms returned to the NVSB and Massey University from 2002 to 2006.

Red dots indicate calculated dose from individual stags that died up to 48 hours after sedation based on stated volume and concentration of the drug used and bodyweight of the stag. Open circles are dose rates estimated for all stags during individual sedation episodes when animals did not die after sedation. Error bars are mean and 95% confidence interval

rate was generally lower than the recommended 0.2 to 0.25mg/kg (“Reversal” packet insert, Phoenix Pharm distributors Ltd) and this is likely to be because the dose rate of xylazine administered was usually lower than the manufacturers’ recommended dose and the animals had been sedated for some time before administration of the reversal agent by which time some of the effects of the xylazine would be expected to be wearing off. The administration of reversal does not appear to be related to stag death. Death occurred in stags that did and those that did not receive reversal. If stag death results from a direct effect of xylazine on the pulmonary tissue it may be mediated through alpha 2-adrenergic receptors on the local cells or by a direct effect of the xylazine on the cells by another mechanism. If alpha-2 receptors are responsible, it would be expected that the administration of an alpha 2 antagonist would compete for these receptors and displace the xylazine, thus reversing the effects on the lungs at the same time as the sedation is reversed. This has been shown in sheep (Eisenach, 1987). This does not appear to be the case when stags have died post-xylazine and suggests that either an irreversible inflammatory response is induced by the xylazine that can not be reversed by yohimbine or that the xylazine had its effect through some means other than binding the alpha 2-adrenoreceptors. For example a direct toxic effect on pulmonary leukocytes. This theory could be further investigated by administering a peripherally acting alpha 2 antagonist that does not act centrally prior to giving xylazine and monitoring the effects of the xylazine thereafter.

Local Anaesthetic

Five of the stags that died were not given local anaesthetic as they were in hard antler or being sedated for Tb testing. One stag died before administration of local anaesthetic, and therefore velvet was removed post-humus. Dose rates of local anaesthetic (Lignocaine 2%) ranged from 15 to 30 mls per animal. No adrenaline was used in local anaesthetic in any case. These findings demonstrate that local anaesthetic use and dose do not play a role in the post-velveting death of stags.

Conclusions

Stag deaths appear to be clustered on certain properties and at certain times on that property. Deaths occurred either immediately after sedation or between 6 and 48 hours after sedation. This study was not able to identify the exact risk factors that lead to post sedation stag death in certain animals.

Some factors appear unlikely including the formulation of the drug, dose rate administered, whether or not reversal agents were given, apparent level of stress in the animals, age of animals, time of day and weather conditions.

The most common finding in stags that died was pulmonary oedema, often with evidence of inflammation. Observations of the direct effects of xylazine and other alpha 2 agonists and antagonists on pulmonary function in normal and at risk animals would be a better way of studying this further rather than relying on case report data submissions.

5.2 Hypoxaemia in weaner red deer following sedation with xylazine.

5.3 Observation of yearling stags sedated with “Fentazin” (fentanyl-azaperone-xylazine combination) and given oxygen during sedation

These chapters have been moved to Appendix 5

5.4 Observation of adult stags sedated with low dose xylazine and remaining standing during velvet antler removal

Introduction

As far as the author is aware, no studies have been done to date investigating the hypoxaemic effect of xylazine in deer that remain standing during light sedation. This study measured the haemoglobin oxygen saturation of mature red stags during sedation for velvet removal on a commercial farm.

Materials and Methods

Five adult stags were sedated with xylazine for velvet removal on a farm in Timaru. A dose rate of 0.1 to 0.25mg/kg was administered intramuscularly by the farmer and the stags were allowed to settle until they were drowsy. This took approximately 10 to 15 minutes.

All stags remained standing with head held at or below shoulder height.

A pulse oximeter (Nellcor N65 handheld pulse oximeter, Covidian-Nellcor, USA) was placed on the ear of each stag until a reading was obtained. SpO₂ and heart rate were noted for each animal.

Results

SpO₂ and heart rate recordings are shown in Table 5.4.

Table 5.4 Pulse oximeter SpO ₂ and heart rate readings from 5 adult red deer stags during standing sedation with xylazine for velvet removal		
Stag	SpO ₂	HR
1	84	76
2	74	60
3	84	46
4	85	35
5	90	67

Discussion

SpO₂ readings from standing, lightly sedated deer were decreased from expected normal levels of at least 95% and all were 90% or lower indicating a degree of hypoxaemia. As these animals remained

standing, ventilation-perfusion mismatch due to recumbency was not the cause of shunting. The SpO_2 values were similar to some deer that were sedated in Chapter 5.2 that were administered a much higher dose of xylazine and were recumbent during monitoring. Respiratory depression or pulmonary shunting due to other means may be responsible. It is also possible that the readings obtained in this study using the pulse oximeter were inaccurate due to peripheral vasoconstriction at the level of the ear. Readings were not taken until 10 to 15 minutes after injection of xylazine by which time the initial vasoconstriction is likely to have subsided. Unfortunately pre-sedation sampling in adult stags is not practical.

Conclusion

Haemoglobin oxygen saturation is reduced to levels of or below 90% in stags that are sedated with a low dose of xylazine and remain standing. Ventilation/perfusion mismatch due to recumbency is not responsible for this decrease in oxygen exchange.

Discussion of Chapter 5

Post-velvetting or post-xylazine stag deaths continue to occur in the deer industry more than 20 years after they were first described in the literature by Walker and Middleberg (1988). Survey data found that approximately 1/1000 sedations with xylazine or xylazine combinations results in death within 48 hours. It has been estimated that approximately 110,000 stags are velveted per year with 45,000 of these also having regrowth removed (T. Pearse, pers comm) and that approximately 50% of stags that are velveted are sedated (A. Conway, NVSB, pers. comm). This would result in around 50 to 100 stag deaths per year across the country. Deaths are sporadically distributed in time and location with some farmers experiencing multiple deaths, some never experiencing a death after years of velveting stags and others losing one or two. The perception of the degree of the problem thus varies between farmers. Most deer veterinarians have had experience with post xylazine deaths on their clients farms.

This study was not able to identify specific farm related risk factors but it appears that drug formulation, dose rate and method of use are not important factors. Animal handling, facilities, temperature and weather conditions also appear to be unrelated to stag death. Individual animal susceptibility to xylazine appears to be the most important factor and this may be breed or genetics related or due to some underlying pathological condition, possibly of the respiratory system.

The most obvious postmortem finding from stags that have died within 48 hours of xylazine has consistently been pulmonary oedema. This has sometimes been preceded by observed respiratory distress. This has led to the assumption by veterinary clinicians that the reaction is a hypersensitivity or allergic reaction to the drug. While clinical, postmortem and histological findings do have certain characteristics of a hypersensitivity reaction, deaths have occurred in animals that have never been exposed to the drug before. It may be better to describe animals as being particularly susceptible (or sensitive) to xylazine but the involvement of an immune mediated response is uncertain. It is quite likely that acute death involves an acute inflammatory reaction not unlike that seen in anaphylaxis. Delayed deaths (6 to 48 hours) may have an inflammatory component or may be the result of an inability of the stag to recover from hypoxaemia or other complications that occurred during sedation. Hypoxaemia during anaesthesia is well documented but the question remains as to whether deaths occur as an extreme outcome of the physiological effects that occur in all stags in response to xylazine or whether susceptible stags undergo a different pathological process not seen in stags that do not die. There was considerable variation between animals of similar age, breeding and farming background in the level of hypoxaemia seen after the same dose of xylazine had been administered (Appendix 5.2, 5.3) lending support to the theory that deaths occur as an extreme outcome of a common reaction to xylazine.

Histological examination of the lungs may be useful in understanding the pathophysiology of the disease. Slightly different results have been obtained from different studies. While pulmonary oedema has been a consistent finding, often with haemorrhage, the types of white cells infiltrating the lungs has differed. Walker and Middleberg (1988) found marked infiltration with degranulating eosinophils in stags that had died but not in stags that had been sedated with xylazine and subsequently recovered. This was not a consistent finding from the postmortem samples that were

sent to Massey University which tended to have increased numbers of neutrophils or lymphocytes but not eosinophils. It is still unclear whether these cellular changes indicate an underlying pathological condition such as lungworm or pneumonia that increased the risk of complications during and after sedation or whether they were induced by the xylazine *per se*. None of the histological reports from deer in the current studies have indicated changes in pulmonary intravascular macrophages. These cells have been shown to be affected by xylazine administration in sheep and are present in normal deer lungs (discussed further later). It is also unclear what the relationship is between the cell types found in the pulmonary parenchyma and the effects of xylazine on circulating white blood cells. *In vitro* studies showed that xylazine caused disruption of basophils corresponding with an *in vivo* decrease in circulating basophil numbers that returned to normal by one hour (Cross et al, 1992). It is possible that cellular damage to basophils or eosinophils within the lung tissue causes degranulation and release of histamine, triggering a local inflammatory response and chemotaxis of other types of white blood cells such as lymphocytes.

Hypoxaemia has been well documented in ruminants, including various species of deer, sedated with opioids, alpha-2 agonists or, more commonly, combinations of these. The results from the current study support this and show that marked hypoxaemia occurs in all young red deer that are sedated with xylazine alone and do not die post sedation. This was measured using pulse oximetry and arterial blood gases. As pulse oximetry is cheaper and technically easier to perform and provides continual monitoring it is a useful tool for monitoring deer during sedation when a veterinarian is performing the procedure. This tool is not readily available to most farmers. The accuracy is likely to be reduced below 70% saturation (according to the manufacturers specification) and problems may arise when there is vasoconstriction or poor circulation to peripheral tissues. At saturation levels below 70% animals should be reversed from sedation or administered supplemental oxygen anyway, so readings below this may indicate severe respiratory compromise but the response should be the same. Caulkett and Haigh (2004) recommend monitoring oxygen saturation levels in all sedated wildlife with a pulse oximeter and providing nasal insufflation of oxygen when SpO₂ is less than 85%. These authors highlight the potential complications of hyperthermia with hypoxaemia and the potential for fatal capture myopathy. Normal oxygen saturation in captive wildlife when measured with a pulse oximeter is 95 to 98% according to these authors. Nasal insufflation of oxygen produced marked improvements in both SpO₂ and PaO₂ and tracheal supplementation may provide even greater increases. Medical air did not sufficiently improve oxygenation in sedated deer.

Hypoxaemia develops when there is a ventilation/perfusion mismatch or pulmonary shunting. Some or all of the blood circulating through the pulmonary vasculature is not able to achieve full oxygenation before returning to the heart and thence into the general circulation. This may be brought about due to decreased volume or concentration of inspired oxygen, decreased ability of the chest wall to expand, for example due to recumbency and pressure from internal organs, vasoconstriction in the pulmonary vasculature, or thickening of the alveolar walls or interalveolar septa, for example due to oedema or inflammation, thus increasing the diffusion barrier or accumulation of blood and/or proteinaceous fluid (Tranquilli et al, 2007).

It is likely, but not yet proven that the hypoxaemia and the pulmonary oedema that have been observed in these studies are related and that some degree of pulmonary parenchymal change

occurs in all red deer that are sedated with xylazine with death occurring only when the changes are so severe that the animal is unable to compensate. Further research is required to understand the mechanism of these effects and determine whether pre-treatment with other drugs is likely to mitigate the effects of xylazine, thus reducing the risk of death. Slaughter trials whereby animals are postmortemed during xylazine sedation with various treatments may be useful but are costly and may not be accepted by animal research ethics committees. It would be preferable to examine the effects by indirect measures without having to euthanase the animals. Pressure within the pulmonary vasculature or right ventricle, pressure within the airways, bronchioalveolar lavage or pulmonary biopsy may be methods to further investigate the effects of xylazine and other treatments in deer.

Most research has focused on understanding the mechanism of this condition in sheep. Deer may develop hypoxaemia by a similar mechanism to that seen in sheep.

Poor ventilation due to recumbency is unlikely to be a major contributing factor. Sheep given a low dose of alpha 2 agonist and maintained upright in a sling develop severe hypoxaemia (Celly et al, 1997; Waterman et al, 1987). The peripheral alpha 2 agonist ST-91 causes hypoxaemia without sedation in sheep (Celly et al, 1997, 1999b). SpO₂ readings in 5 lightly sedated standing sedated stags (Chapter 5.4) were all 90% or lower with the lowest being 75%. Some deer that are heavily sedated and recumbent are able to maintain relatively high SpO₂ levels, even when tightly strapped into a cradle for CT scanning (Appendix 5.2).

Hypoxaemia in sheep appears to be mediated by peripheral alpha2 adrenergic receptors. Xylazine, detomidine, medetomidine and ST-91, an alpha 2 agonist that does not cross the blood brain barrier all induce hypoxaemia after IV or IM administration. The hypoxaemic effects of clonidine are eliminated by administration of alpha-2 antagonists, but not alpha-1 antagonists (Eisenach, 1988). Injection of alpha 2 agonist into the cisterna magna causes sedation, but not hypoxaemia (Nolan, 1986). Administration of idazoxan five minutes before either xylazine or detomidine abolished hypoxaemia (Waterman et al, 1987). Idazoxan and tolazoline significantly decreased the duration and severity of hypoxaemia in sheep when given to sheep five minutes after xylazine IV (Hsu et al, 1989). Injection of tolazoline or yohimbine caused an immediate and significant improvement in SpO₂ in the deer in Appendix 5.2.

Eisenach (1988) suggested that platelet aggregation may be responsible for ventilation-perfusion mismatching (pulmonary shunting). Nolan et al (1990) tested this theory and found that administration of aspirin before giving xylazine decreased thromboxane levels by 95% but had no effect on the level of hypoxaemia induced by xylazine. Xylazine failed to induce platelet aggregation *in vitro* in this study. Circulating platelets may be reduced in response to xylazine and this is likely to be, at least in part, due to splenic sequestration by the same mechanism as decrease in red blood cell concentration (Mackintosh and Cross, 1989).

Xylazine may trigger an acute inflammatory response resulting in pulmonary oedema and increased oxygen diffusion barrier. This could be brought about by activation of proinflammatory cells such as pulmonary intravascular macrophages (PIMs).

Sheep that were given clinically sedative doses of xylazine demonstrated severe pulmonary parenchymal damage and morphological changes to pulmonary intravascular macrophages was evident at 3 minutes, 10 minutes and 60 minutes after administration. Changes were virtually restored to normal after 12 hours. ST-91, also caused damage to capillary endothelium resulting in pulmonary oedema three minutes after administration, without signs of sedation in the sheep (Celly et al, 1999a).

Activation of PIMs may result in the release of several proinflammatory mediators. PIMs in horses can be activated by vascular tracers and halothane and possibly endotoxins. Horses depleted of PIMs using gadolinium chloride demonstrated lower mean pulmonary arterial pressure and less interleukin 1 positive cells compared to normal horses when challenged with *E. coli* lipopolysaccharide (Parbhakar et al, 2005). Pulmonary Intravascular Macrophages can be permanently destroyed with dichloromethylene-diphosphonate. (Rooijen and Nieuwmegan, 1984). A less toxic method of inhibiting intravascular macrophages is the use of detergents. Staub and coworkers (2001) have shown that the relatively inexpensive triton polymer, tyloxapol, inhibits the response to endotoxin and decreases phagocytosis by PIMs. In that experiment, sheep administered endotoxin showed an increase in plasma and lymph TNF that peaked at 1 hour after infusion. Tyloxapol given prior to LPS decreased this response by 90%. Using this detergent, pulmonary and systemic inflammatory responses to endotoxin decreased, but the macrophages still phagocytosed monastral blue (indicating they remained functional). Giving detergent to horses resulted in decreased rise in pulmonary arterial pressure, reduced leukopenia and abolished increase in rectal temperature in horses that were given *E. coli* lipopolysaccharide (Longworth et al, 1996). It is suggested that these physiological responses to endotoxin are induced by inflammatory mediators and that inhibition of PIMs prevents the release of these mediators. Interestingly horses do not suffer from significant hypoxaemia during sedation with alpha-2 agonists.

Pulmonary intravascular macrophages have been identified adhered to the endothelium of pulmonary capillaries in deer. (Carrasco et al, 1996). Electron microscopy revealed these to be distributed throughout the lungs, about twice as abundant as alveolar macrophages and about half the cross sectional area. They had numerous tubular invaginations of the membrane. Activation of PIMs by xylazine may cause release of intercellular mediators that trigger pulmonary endothelium injury with increased microvascular permeability and consequent interstitial and alveolar oedema and accumulation of neutrophils. Alveolar macrophages recovered from 3 wapiti deer and by bronchoalveolar lavage following sedation with xylazine were intact and did not show signs of cellular damage (Silflow and Foreyt, 1994). This suggests that if macrophages are involved, the intravascular macrophages may respond differently than alveolar macrophages. The potential involvement of PIMs warrants further investigation but is challenging without running slaughter trials as it appears that alveolar lavage may not be a successful means of recovering the cells in the live animals. Pulmonary biopsy could be considered. Another option is to look for evidence of acute inflammatory mediators or late stage inflammatory mediators. Some potential mediators include TNF- α , haptoglobin, histamine and interleukins. Recent advances in genetic research may allow for identification of gene sequences or gene expression rates in stags that are susceptible to the effects of xylazine.

If pulmonary oedema and hypoxaemia result from cellular damage and inflammation within the

lungs, this must be at a level that is able to be overcome by both oxygen supplementation and the administration of an alpha-2 antagonist considering that both of these interventions have been shown to significantly improve oxygenation, even in severely hypoxaemic deer.

Understanding the mechanism of post xylazine stag death may help to develop a solution to the problem and may also help in the wellbeing of all deer that are sedated with xylazine and potentially suffering from respiratory compromise. Alpha-2 agonists are very useful for restraint for velveting and other minor procedures in deer so maintaining their use while decreasing the side effects is desirable. If hypoxaemia is purely an alpha-2 agonist effect and a peripheral alpha-2 antagonist that does not cross the blood-brain barrier could prevent hypoxaemia this may present a simple solution. Such a drug may be given prior to, in combination with or immediately following the administration of xylazine. A potential peripherally acting alpha -2 antagonist that does not alter the sedative effects of clonidine in humans (Warren et al, 1991) and reverses the cardiovascular side effects of dexmedetomidine in dogs (Honkavaara et al, 2011) is MK-467.

There may be differences in the effects of different alpha 2-agonists in deer with some potentially being safer than others. This requires further research in deer before use of alternative alpha 2 agonists can be recommended. For example detomidine appeared to produce less hypoxaemia compared to xylazine in the observations in Appendix 5.2. This may be related to the relative selectivity of the drugs for different subtypes of alpha 2 receptors or actions at other receptors. Another possible explanation is that because detomidine is approximately 100 times more potent than xylazine (Schwartz and Clark, 1998a), the absolute dose of drug is lower. Detomidine has a high affinity for alpha 2 receptors compared to alpha 1 receptors at low -moderate dose rates (Virtanen and Nyman, 1985) but did not show selectivity within alpha 2 receptor subtypes A, B, C or D (Schwartz and Clark, 1998a). In a comparative study, medetomidine had a higher alpha 2:alpha 1 ratio affinity compared to detomidine, clonidine UK 14,304 and xylazine with ratios of 1620, 260, 220, 300 and 160 respectively (Virtanen et al, 1988). Despite this, Celly et al (1997) found that equipotent sedative doses of xylazine, detomidine, medetomidine and romifidine produced similar levels of hypoxaemia in sheep indicating that alpha 1 effects are not responsible and that the absolute dose of drug does not affect the level of hypoxaemia in sheep when equipotent doses are administered. The cardiopulmonary effects of medetomidine and xylazine were similar in calves given equipotent doses intravenously with similar levels of hypoxaemia developing with both drugs although the effects on blood pressure were different for the two drugs (Rioja et al, 2008).

Use of highly selective alpha 2 agonists and antagonists medetomidine and atipamezole may be preferable to the use of xylazine and yohimbine. It is worth considering the longer duration of action of detomidine and medetomidine compared to xylazine which may result in further delayed complications, especially if the reversal agent is given intravenously and is eliminated faster than the intramuscular dose of the agonist agent that was delivered intramuscularly. Other drawbacks of using detomidine or medetomidine include the low concentration of the drug in commercially available formulations, necessitating a large volume that is not practical to deliver into the muscle using a pole syringe and the relatively high cost of these drugs compared to xylazine.

Relying on postal surveys and post-incident veterinary reports was an unsatisfactory means of gathering data. This is most likely because the time involved in completing the forms and sending

them was prohibitive in the setting of a busy practice. Nevertheless, it would still be very useful to gather further postmortem and histology reports from stags that had actually died following sedation. This could be done through a simplified process of information and sample gathering or through gathering information directly from the veterinarians normal laboratory.

Conclusion

Post xylazine stag deaths are a recognised condition that continues to occur at a rate of around 1 per 1000 sedations. We have shown that severe hypoxaemia, of a level indicating oxygen supplementation should be provided, occurs in most red deer administered xylazine at 0.5mg/kg intravenously. The range in responses was large suggesting marked individual variability in response irrespective of breed, age, environment and dose given. It is likely that the mechanism responsible for hypoxaemia also causes post xylazine death. An acute inflammatory response involving release of pro-inflammatory mediators and late stage inflammatory mediators appears to be a plausible explanation at this stage. Such a response may be triggered by damage to pulmonary intravascular macrophages and mimic severe inflammatory disease due to sepsis. Further investigations should include the release of inflammatory mediators after xylazine sedation or the effect of administering inflammatory inhibitors on the hypoxaemic effects of xylazine.

Xylazine may cause more severe hypoxaemia than detomidine, and xylazine in combination with an opioid (e.g “Fentazin”) is likely to cause more severe hypoxaemia than xylazine alone.

Although survey results show that stags given alpha 2 antagonist to reverse sedation are still at risk of post-sedation death, it has reversed the hypoxaemia in this study and in sheep in other studies and is therefore recommended for all deer given xylazine.

Hypoxaemia appears to be mediated by peripheral alpha 2-adrenergic receptors and administering a peripheral alpha 2 antagonist prior to or during sedation with xylazine may help to overcome this.

Chapter 6

General Discussion and Future Research

This thesis has examined several different aspects of velvet removal from red deer in New Zealand. Methods used for farming deer and harvesting velvet have progressed a long way since deer venison and velvet were first realised as a valuable export product and deer were trapped from the bush. Considering the size and age of the deer farming industry in New Zealand, there has been a reasonably large amount of research done relating to velvet removal and sedation of deer. This has been enhanced by research conducted in Canada, mainly of wapiti and white tailed deer. The various experiments that make up this thesis have, for the most part, been a continuation of previous research, hopefully providing some answers to some of the questions previously raised but also highlighting areas of future research and improvement.

On the method of administering local anaesthetic

The ring block method had previously been shown to be superior to individual local site nerve blocks (Wilson et al, 1999; 2000) but it was evident that variability in the analgesic efficacy still existed when used in the field (Morrow and Matthews, 1998; Conway, 2005). The results of the observational study in Chapter 2 showed that a continuous line of overlapping injections of local anaesthetic was the most important aspect of the ring block method. When gaps occurred in the ring block on the cranial, lateral or caudal sides of the pedicle, the stag was more likely to demonstrate a withdrawal response. The results from this project emphasise the importance of careful placement of each injection.

Future research on velvet antler analgesia

The repeatability and reproducibility of the method (a complete ring of overlapping injections of local anaesthetic) should be tested by training a number of farmers to use the method and measuring the analgesic success using a behavioural withdrawal response as an indication of failed analgesia. A minimum of 20 stags per farmer would be desirable and having several farmers perform antler analgesia on stags on the same property would be preferable to minimise confounding factors.

The observational study suggested that it may be more difficult to achieve analgesia in older stags and in stags that are in the regrowth stage of velvet growth. Wilson et al (2000) also considered that regrowth antler may be more difficult to block reliably. These questions should be further investigated using controlled experiments to remove the confounding that precluded robust statistical analysis in Chapter 2.

Repeatability of analgesic success may also be improved by use of a tool that can deliver local anaesthetic more consistently and reliably such as a needle free injection (Mitragotri, 2006) wheel. This could be run around the base of the pedicle delivering a small volume of local anaesthetic every few millimetres without having to use a needle. A device like this may also decrease the amount of lignocaine residue in the antler because there would be less chance of injecting directly into an artery supplying the antler. In Chapter 4.6, casts of the antler revealed these arteries to be extensive, large and overlapping right at the position that local anaesthetic is applied in the ring block. It is quite possible that arterial spike contributes to high concentration of lignocaine in

some antlers as demonstrated in Chapter 4.

Articaine may prove to be a better local anaesthetic than lignocaine as it is preferred for dentistry and had better bone penetration (Johansen, 2004) and provides more reliable analgesia than lignocaine. A controlled experiment comparing the behavioural response of adult stags to articaine and lignocaine, based on the behavioural scoring system in Chapter 2 should be done to determine the efficacy of Articaine for providing analgesia for velvet antler removal.

On the method used in Chapter 2 - Uncontrolled observational study

In the study described in Chapter 2, data were collected using video recordings of farmers in the field velveting their own animals, and these videos were analysed retrospectively. It was not a controlled experiment and resulted in a large number of independent variables. This method has advantages and disadvantages. To determine which factors are important to analgesic success we aimed to identify why some farmers achieve better results than others. Therefore, it was appropriate to observe farmers administering the ring block in the field. The major disadvantage of the method used was the high degree of confounding in the data. Statistical analysis of the results was challenging and often unrewarding because there were so many variables under investigation at one time and each farm was quite different from the others in farm management practices, type of animals, and the injection method used by the farmer. The ideal statistical analysis would have been a multivariate analysis that would be able to determine the most significant aspects that contributed to the success of the lignocaine ring block. This was not done because of the high level of confounding and the likelihood that the results would be misleading. Multivariate analysis creates new “variables” that may not have a practical meaning with respect to application of the local anaesthetic.

The method used was relatively inexpensive as animals did not need to be purchased and kept for the trial, a large number of velvet removal operations could be investigated, a large cross section of animal types, ages, genetics and farming backgrounds could be included in the analysis and in most cases, only one researcher was required to gather the data and perform the analysis at a later time.

The lack of robust statistical data means that there are still several unanswered questions about the importance of many of the variables assessed. To definitively determine the importance of these, a controlled experiment would be required to ensure that only one variable is under investigation at a time. This would require a large number of animals and the costs and time required would be substantial.

On Behaviour to assess a pain response in deer

One difficulty experienced in using an acute withdrawal response as an indicator of analgesia failure during velvet antler removal was the differences in normal behaviour between stags. This was most apparent when stags from different farms were included in the same experiment as not only

was there variation between individual animals but a clear difference was seen between groups of animals from different farms of origin (Chapter 3.2). To my knowledge, this marked difference in withdrawal behaviour between animals from different backgrounds and upbringing has not previously been described.

The stags that were raised on the Massey University farm tended to struggle more during restraint and particularly during injection of lignocaine. They appeared to be more sensitive to the pain associated with needle prick and injection of the drug which is known to cause stinging in some people. These stags also moved more during cutting of the antlers regardless of the analgesic used. This was despite the same behaviour scoring criteria as that used for adult stags in Chapter 2. The group of bought in stags used in the *NaturO*TM ring study showed quite different behaviour and tended to stand quite still throughout the entire procedure. These bought in animals were generally more flighty and agitated during mustering and yarding and it seems that this contributed to their response during handling which suggests that a “freeze” response or standing very still in the crush is more common in animals that are experiencing a greater level of fear or stress than in those that are more accustomed to people and the handling facilities. This has also been observed (by the author) in young deer and adult hinds on the Massey Deer Unit. Those that are handled and blood sampled regularly are generally very quiet to muster and easy to work in the yards but will struggle violently in response to jugular venipuncture and some hinds will bite the handler. Animals that are more jumpy during mustering tend to be less likely to show this sort of response during venipuncture. It may be that the stress experienced by the more agitated animals causes some analgesia so that they do not “feel” the injections and possibly even the saw cuts. Nicol et al (2009) found that around 30% of yearling stags that had a nick test (light saw cut) to the antler did not show a behavioural response, whereas others showed a marked behavioural response to the nick test. This may be because these animals were more highly stressed and either did not feel the pain associated with the saw cut or may be that the response of these stags to the experience of pain was to freeze rather than move. A response that is, in effect, no response, is very difficult to measure.

We used a different approach to assessing behaviour as a means of determining a pain response in a controlled, blinded study on the effect of *NaturO*TM rings during one hour of application on the antler pedicle (Chapter 3.4). In that experiment, behaviour was captured on video and behavioural ethograms were developed and analysed with the software, JWatcher. I consider video capture of behaviour data to be superior to live scoring of behaviours and should be standard procedure for this type of investigation. In previous studies, live scoring has been used to determine the difference between stags that were and were not velveted (Pollard et al, 1992b). Although a large number of variables were studied, few differences were observed. In my experience, it is quite difficult to live score behaviours in deer as the observer must stop looking at the animal to write on a recording sheet and is likely to miss several behaviours. Alternatively, the scorer can call out to a person that is recording the behaviour and this is likely to alter the behaviour of the deer in response to the human voice. It is also impossible to double check the behaviour and whether it has been accurately recorded after the event. In the study in Chapter 2, live scoring was attempted initially but it quickly became evident that scoring off the video capture would be more reliable and more objective. Using the video captured in the *NaturO*TM ring study meant that a technician could be trained to observe and record behaviours directly into JWatcher. This technician was

blind to the treatment and did not participate in the direct handling of the animals and therefore did not have any anticipation of the types of behaviours that would be seen. This allowed for a relatively unbiased set of behaviours to be identified along with some unique behaviours that had not previously been described in deer. The results showed that common behaviours such as walking, pacing, turning the head, grooming etc were not affected by treatment but that these unique behaviours that included head rotations and arching the head up and down were more likely to predict animals that had been treated with *NaturO*TM rings. The other major advantage of using the video capture was that the presence of humans could be completely eliminated. This was important as the presence of a human, potential predator, is likely to alter the behaviour of deer.

These studies have highlighted the importance of using behaviours that are specific to the stimulus being applied and the group of animals that are being observed. Young animals may respond differently than adult animals to the same noxious insult and the response to application of *NaturO*TM rings may be different to antler cutting and this may also be different to electrical stimulation of the antler. Using video capture and behaviour recording software without a preconceived set of behaviours in mind should be standard. The behaviours to be analysed should be determined during initial video observation but the behaviour scorer should be blind to the treatment if possible. It is also important that the behaviour scorer remains observant to the occurrence of new, possibly quite subtle behaviours. Using the behaviour software, JWatcher allowed for new behaviours to be added after the initial behaviour files were set up which was an advantage and a feature not available with some other software. On later watching a section of video I observed a behaviour that involved a stag rubbing its chin on the back of another stag. This was repeated by other stags on different tapes but this behaviour was not noted by the technician that did the behaviour recording for the full data set. While it will never be possible nor desirable to identify every single behaviour this does demonstrate that there remains some subjectivity in behaviour scoring even using a controlled method with video capture. It is my opinion that all behaviour analysis studies in deer should be done using video capture without the presence of a human observer and preferably without external disturbance. Ideally this would be indoors and the deer would have been previously habituated to the environment.

Future research on behaviour in deer

More research into specific behavioural responses of deer to certain stimuli is required. This may require high definition video cameras to be strategically positioned so that subtle behaviours can be identified. Ideally, behavioural responses to repeated stimuli would be recorded so that the sensitivity and specificity of each behaviour as a response to a particular stimulus could be calculated. A difficulty with this type of research is that repeated noxious stimuli should not be inflicted on the conscious animal if it is likely to cause distress or fear. Another approach may be to undertake detailed video observations in the field and record the response of deer to naturally occurring stimuli. This approach adds the increased variability due to confounding environmental factors and a difficulty in determine which stimuli deer may be responding to.

In the future if behavioural studies that aim to assess the acute withdrawal response to antler cutting are to be conducted on stags, it is recommended that controlled experiments be done rather than observational studies and that animals from one farm only are used. It is also recom-

mended that stags are sedated with a drug such as zuclopenthixol to reduce the confounding of background stress-related behaviour (Read et al, 2000; Woodbury et al, 2001, 2002). It will always be important to extend the research into the farm situation as this is where the technique is to be applied and the research is worth little if it can only be repeated in the laboratory and will not benefit the welfare of stags on the farm or the quality of the product.

Although most experiments investigating the behaviour of calves and deer following dehorning or velveting have used eight to twelve animals in each treatment group, our data suggest that there is a large variance in behaviour data and this can not be determined before the study as different cohorts show a different behaviour pattern. Treatment groups should be as large as possible but preferably with a minimum of 20 to 30 animals per treatment.

On the use of EEG

EEG had been used previously to assess a response to antler removal and application of high pressure compression bands in stags (Johnson et al, 2005). All other published studies assessing the effect of noxious stimuli on EEG have been conducted in different species and tissue types that are unlike the antlers of deer. Gibson et al (2007) studied the EEG changes in response to scoop dehorning in calves which could be considered somewhat similar to the removal of antlers from deer. In the studies in this thesis (Chapters 3.1 and 3.3), the EEG response to antler removal when no analgesia was applied, were similar to those that shown by Johnson et al (2005) and Gibson et al (2007), confirming that the changes in the EEG pattern in response to an acute noxious stimulus are repeatable across different studies, different types and ages of deer and even different species. Similar changes in TP and F50 were seen following castration in ponies (Murrell et al, 2003) and electrical and thermal stimulation of the tail of rats (Murrell et al, 2007) but these studies did not show any changes in F95.

Depth of anaesthesia is maintained at the same level by adjusting the intake of anaesthetic agent to maintain a constant end tidal concentration of anaesthetic agent throughout the procedure. This is a necessarily light plane of anaesthesia that allows the animal to respond to a noxious stimulus and light enough that a physical response may be seen as was demonstrated in a proportion of animals that had high pressure compression bands applied by Johnson et al (2005). If a physical response does occur, it causes interference with the recording, tending to greatly increase the total power during the time that the movement is occurring but has lesser effects on the median frequency and spectral edge frequency. Movement associated with applying the analgesic and cutting the antler had similar effects on the EEG recording and this section of data is generally not analysed. The equipment used is quite sensitive as the voltages detected are small. Interference from external electrical equipment can cause a lot of noise in the recording and contact between the animal and earth (as we found when saliva drained from the deer's mouth and made contact with the ground) can also cause interference. A Faraday cage is often used in the laboratory to isolate the electrical signal from surrounding electrical equipment in the building. On the deer farm we found that there was relatively little background electrical noise and that the signal was quite clear most of the time.

Another question with the use of EEG is the effect of anaesthetic drugs. Halothane, propofol

and ketamine effects have been studied in various species (Murrell and Johnson, 2006). Propofol blunts the EEG response to electrical stimulation of the tail of rats (Orth et al, 2005) Ketamine and propofol are both likely to affect the EEG. We waited a minimum of 30 minutes after induction of anaesthesia to allow the concentrations of these short acting drugs to be greatly reduced and thus their potential effects to be negligible at the time of recording. The effect of lignocaine could not be eliminated as it was necessary under the treatment protocols to inject lignocaine shortly before cutting the antler. In the study in Chapter 3.4, the response to injection of lignocaine was interesting and resulted in significant changes in the EEG frequency spectrum that were different from those seen in response to an acute noxious stimulus. It appears that lignocaine had a centrally acting effect in the EEG. This raises questions as to the effect of lignocaine itself on the EEG response to cutting the antler. It may simply be that the lignocaine causes a shift in the baseline and that changes immediately before and after antler cutting are still comparable. This could be tested by injecting lignocaine intravenously prior to cutting an antler that had not been provided with local analgesia. In the EEG studies in this thesis animals were not tested for an EEG response prior to admission for the study. In hindsight this would have been a good idea. A proportion of animals that had their antlers removed without any local analgesia did not show a significant change in the EEG pattern. It was speculated that a similar proportion of animals in the other treatment groups would also not have shown a response although the exact proportion in each treatment are likely to be different due to the small numbers of animals in each group. Excluding non-reactors prior to the study would have allowed for more reliable statistical analysis and this should be done at the start of any EEG study on the response to noxious stimuli in the future.

On *NaturO*TM rings for analgesia

*NaturO*TM rings continue to be an accepted form of analgesia and are widely used on spiker deer for velvet removal prior to transport to slaughter. Overall, it appears that compression of nerves using this medium pressure compression band impedes conduction along at least some pain fibres and especially fast conducting fibres. This is supported by the decreasing response to antler electrical stimulation following the application of a *NaturO*TM ring shown by Nicol et al (2009) and the reduced EEG response to velvet antler removal after a *NaturO*TM ring had been applied compared to no analgesia seen in Chapter 3.1. The ability of the *NaturO*TM ring to reduce the EEG response to velvet removal was not as great as that seen with lignocaine ring blocks but was not statistically significantly different from either lignocaine or controls. The number of deer included in each treatment group was not large enough to determine whether *NaturO*TM rings were more similar to lignocaine or no analgesia (control). This could have been overcome by including a larger number of deer in each treatment or by removing deer that did not generate an EEG response to noxious stimulus.

A proportion of animals that had *NaturO*TM rings applied (Chapter 3.4) demonstrated some unique behaviours at a higher frequency than those with no *NaturO*TM rings applied. These behaviours tended to occur at a high frequency during the first 10 minutes and were reduced to near baseline levels by 30 minutes. This time-frame corresponds with the decreasing response to electrical stimulation of the antler over time (Nicol et al, 2009).

A major limitation of the experiment in Chapter 3.4 was the small number of animals in each treatment group and large variation in behaviours between animals. The number used (ten) was similar to other studies in deer and cattle. Pollard et al (1992, 1993, 1994) consistently used eight animals per treatment to investigate the behaviour of deer following velvetting with different analgesic treatments.

Overall, it appears that *NaturO*TM rings are effective at preventing a behavioural response to antler cutting but that a proportion of animals may experience some unpleasant sensation on application of the rings, especially during the first 10 to 30 minutes. This could be overcome by injecting lignocaine below the *NaturO*TM ring immediately after it has been applied. This should only be done if it can be guaranteed that lignocaine residues will not occur in the meat if the animals are to be sent directly to slaughter and the buyer of the velvet should also be aware that lignocaine has been used in the animals and that leakage past the level of the *NaturO*TM ring into the velvet is a possibility.

Future research on *NaturO*TM rings

It is uncertain whether all of the pain conducting fibres travelling from the antler to the brain would be fully blocked by the *NaturO*TM ring, particularly whether slow conducting, unmyelinated C fibres are effectively blocked. Delayed and prolonged changes were seen in the EEG after antler removal in some stags that had previously been treated with a *NaturO*TM ring and a possible explanation for this is that C fibres may have maintained function whereas A-delta fibres were effectively blocked. This would be best tested by using nerve conduction studies whereby nerves are stimulated above the site of the *NaturO*TM ring and a response recorded in nerves below the *NaturO*TM ring. Assessing times between stimulus and recorded response would allow determination of the type of nerve fibre that is conducting.

While *NaturO*TM rings appeared to prevent a behavioural response to velvet antler removal (Nicol et al, 2009), the behavioural study in Chapter 3.2 may not have been appropriate as the *NaturO*TM ring might prevent an acute withdrawal response but pain may be experienced in the seconds and minutes after antler removal. This is an important question that needs to be addressed and the best way to do this would be a behavioural study, similar to that described by Webster and Matthews (2006), comparing the behaviour of stags for 60 minutes (or longer) after antler removal following *NaturO*TM ring application or lignocaine ring blocks and comparing this to behaviour for 60 minutes prior to velvet antler removal and behaviour of deer that do not have antlers removed.

On lignocaine residues in velvet antler

The experiments in Chapter 4 of this thesis failed to develop a method that would reliably prevent lignocaine residues in velvet antler. Some antlers had lignocaine residue more than 100 times greater than the current MPRL of 0.1mg/kg. The acrylic arterial cast (Chapter 4.6) showed that the number and pattern of arteries crossing the antler pedicle at the level of injection of local anaesthetic was considerably greater than originally described by Adams (1979). The course of these blood vessels across the pedicle was complex with arteries often seen stacked on top of each

other. Cross sections of the antler pedicle revealed irregularities in the bone surface which was covered with an connective tissue of variable thickness. Within the connective tissue blood vessels were irregularly located. It is likely to be difficult to apply a consistent pressure around the entire pedicle that is able to completely occlude all of these vessels. In the angiography studies (Chapter 4.2), the device used to apply the tourniquet obscured the view of the contrast iodine and had to be positioned out of the way. In one case, this resulted in enough contrast getting past the tourniquet so that it could be seen on angiography. When the device was repositioned and applied at the same tension, no contrast was seen, indicating that the placement of the tourniquet affected leakage of contrast more than the actual tension that was applied on the tourniquet. In the fluorescent microsphere study, microspheres were recovered from all antlers that had a tourniquet applied before injection of microspheres, at all tourniquet tensions, indicating that no matter how tight the tourniquet is, some blood flow to the antler still occurred. In the field study in Chapter 4.7, every antler had lignocaine residue levels above the limit of detection, according to the AssureQuality laboratory, showing that leakage into the antler occurred with all of the types of tourniquet used in that study.

A collateral blood supply, perhaps from within the centre of the pedicle may exist. The acrylic casts made were not able to determine whether such a blood supply exists as the acrylic was injected into the carotid artery about half way up the neck and an alternative supply may branch from more proximal to this site of injection. For lignocaine to reach the antler via this route, it would have to enter the venous blood and be recirculated to the antler. It is unlikely that the high level of lignocaine residue detected in some antlers could be achieved by this means. Another possibility is that blood may back-flow into the antlers through the venous system coming up through the centre of the antler. In chapters 2 and 4 the level of haemorrhage after cutting the antler with the tourniquet in place was observed. In some cases this was quite marked and was a continuous stream of blood flowing from the cut surface. This was thought to be venous blood due to the colour and non-pulsatile flow pattern and the fact that the outer edge of the cut stump was usually not bleeding and the blood flow appeared to come from the centre of the antler. If such a large backflow of blood can occur even when the tourniquet is in place it does lead to the question of whether lignocaine can enter the antler through the venous circulation after systemic distribution. This seems an unlikely explanation however as it would require retrograde diffusion of lignocaine within the venous system and back past valves. Furthermore, the backflow of blood is only likely to occur after the antler is cut as the forward pressure within the antler would prevent this while it is still intact.

Future research on lignocaine residue in velvet antler

A model of the internal vasculature of the pedicle and antler that include the venous structures would enhance our understanding of how blood and drugs circulate through the antler tissue. This could be done use a bone erosion method.

A possible explanation for high lignocaine residues that has not been investigated is contamination of the antler from the hands or equipment of the operator. It would only take a small volume of 2% lignocaine on the surface of the antler to result in high lignocaine residues when calculated as mg/kg of antler. Taking samples from along the length of the antler may reveal differences that could be due to handling contamination of the outside skin. This was done by Bagonluri (2005)

and Wilson (unpublished) but the results were not very conclusive and Bagonluri found that the concentrations of lignocaine tended to be higher at the tip than the base. This could be tested by washing the outside of antler samples or removing the outer hair layer prior to testing for lignocaine residue.

Whatever the cause of the high residue levels in some antlers, it seems unlikely that any further work to develop tourniquet systems is going to be effective at preventing high residue levels. Investigations in the future should focus on the method of application of local anaesthetic and different drugs that do not pose a food safety risk.

A needle free injector (Kent et al, 2004) that delivers local anaesthetic under high pressure immediately under the dermis may prevent noncompliant antler lignocaine residues. This injection technique is unlikely to cause a large volume of local anaesthetic from being injected directly into an artery around the pedicle. A controlled experiment, using a needle free injection device on one antler and standard practice on the other antler with subsequent testing of the antlers for lignocaine residue would be the best approach to determining whether this method reduced residues. The number of antlers tested should be as large as possible, but preferably at least 20 per treatment due to the high variance in lignocaine results.

One drug that shows promise for achieving analgesia without harmful residues is articaine. Articaine is widely used in dentistry and has been found to produce excellent analgesia with better bone penetration than lignocaine and because it has an ester link it is broken down in tissue by local tissue esterases (van Oss et al, 1989; Oertel et al, 1991). The efficacy of this drug for providing analgesia should first be tested in a controlled, blinded study as described above. Development of a new analgesic for velvet removal would also be challenging from a cost and drug licencing viewpoint. The NZFSA would need to take the issue seriously and farming industries and drug companies would have to be willing to invest in the new drug development. If Articaine proved effective and suitable for local analgesia in farm animals it would overcome the current dilemma of needing to provide analgesia without causing potentially harmful residues in food producing animals.

On the effects of xylazine in deer

Xylazine remains a very useful sedative for farmed red deer in New Zealand and has several desirable properties including smooth induction and recovery, predictable, dose dependant sedation, small dose to administer, relatively safe to the operator (compared to opioids), wide safety margin for the animal and it is fully reversible with intravenous yohimbine. There are some drawbacks and the most important of these is the occasional occurrence of death in stags up to 48 hours after administration and the development of acute hypoxaemia in the majority of deer.

The link between the effects of xylazine that we have observed and the occurrence of post-xylazine death is still unclear and warrants further investigation. There may be some other trigger than allows hypoxaemia to develop into death. Perhaps certain animals are unable to compensate for the low oxygen levels during sedation, resulting in fatal tissue damage or perhaps a completely different mechanism occurs in these stags. Regardless of whether there is a direct link between

xylazine-induced hypoxaemia and stag death, the fact that hypoxaemia occurs at a high rate is reason in itself to investigate ways of improving sedation techniques in deer.

Future research on xylazine in deer

Ongoing collection of case-report data could be done to increase the number of reports and statistical power of the dataset. If enough data were collected, risk factors may be identified. For example a genetic predisposition to xylazine reactions may be identified. a simplified survey would be necessary to increase the rate of reporting.

Controlled laboratory studies are more likely to provide useful insights into the mechanism of disease induced by xylazine than epizootological studies.

While hypoxaemia has been measured in deer sedated with xylazine and pulmonary oedema was found in stags that died post-xylazine, the direct link between these has not been established, although it seems likely given the findings in sheep (Celly et al, 1999a). A slaughter trial, with examination of lungs removed from stags at timed intervals after sedation with xylazine is warranted although the cost of such a trial may be prohibitive. An alternative way of assessing the immediate effects of xylazine on the lungs might be to take sequential ultrasound-guided biopsies of lung tissue while the deer is under sedation and examine these histologically. It may also be possible to recover inflammatory cells from the airways of deer under sedation using alveolar lavage.

Hypoxaemia appears to be due to a peripheral alpha 2 adrenergic effect. It would be useful to inject a peripherally acting alpha 2 adrenergic antagonist that does not cross the blood brain barrier prior to giving xylazine to determine whether a drug combination might prevent the hypoxaemic effects of xylazine. MK-467 is such a drug that has been used experimentally (Warren, 1991; Honkvarna, 2011) but is no longer manufactured. Exhaustive efforts have been made to locate a source of this drug without success. It may be possible to manufacture it in-house but the molecule is fairly complex (Professor Michael Harding, pers. comm).

There are similarities between the effects of xylazine in deer and drug-induced pulmonary disease occasionally seen in humans (Kumar and Holden, 1986; Cooper et al, 1988; Rosenow, 1994). Further *in vitro* or *in vivo* investigation into inflammatory mediator release may help determine whether pulmonary oedema occurs as an inflammatory response to xylazine. If so, it may be possible to block certain inflammatory mediators. The expression of inflammatory mediators may be genetically controlled and gene expression studies may be able to identify genetic lines of deer that are more likely to react to xylazine. This could be particularly useful for farmers that wish to know whether a valuable stag is likely to react to xylazine before giving the drug.

There are no other sedative drugs currently available that have the range of useful properties that the alpha 2 agonists have. Alternative alpha 2 agonists to xylazine may be safer as there have been no reports of stag deaths with any of the other alpha 2 agonists although these have not been widely used. It would be useful to further investigate the effects of detomidine, medetomidine and romifidine for sedation for velvet antler removal. Farms that have had significant problems in the past or stags that have demonstrated acute reactions to xylazine could be used to test the potential benefits of these alternatives. While it is unlikely that the deer industry will lead the development

of new sedative drugs, it should pay close attention to other groups that may be involved in the development of sedatives and become involved in research by offering to assist with testing new products in deer.

On the future of velvet removal in New Zealand

Whether velvet removal continues on the same commercial scale as it currently does or whether it continues at all will depend on a number of factors and the most significant of these is likely to be the economics of velvet sales. If velvet continues to be a product in high demand and of high value it is more than likely that its removal will continue. Velvet is also removed from venison stags prior to transport for slaughter and from sire stags to ensure the safety of other deer and the farmer. This will probably continue for some time. It is important that the deer industry is continually reviewing the methods used to ensure that the best available techniques are used and the welfare of the stag is maximised. Painful animal husbandry procedures such as mulesing of sheep to prevent flystrike and tail-docking of cattle can result in trade barriers that are not directly related to the product. In a similar way, velvet removal, if it is perceived to be inhumane could result in barriers on the trade of venison or even of other farm products such as beef, lamb or milk if overseas markets or competitors wish to make a stand against the practice. The deer industry should remain honest and open about the practice of velvet removal and the investment that has been made in research to ensure the most pain free methods with fewest complications for the animal are used. In the future this may include options such as the use of needle free injection devices, alternative local analgesic compounds or methods of local analgesia, new sedatives or additional drugs that counteract the side effects of the current sedatives, the use of long acting analgesics to reduce pain after velvet antler removal and the use of drugs that have not been thought of yet. All of these are likely to add additional cost to the procedure of velvet removal but this should be seen as an investment in quality rather than a compliance cost. What value should be put on the welfare of the animals and the safety and security of our foreign trade?

On this thesis

This thesis has been a large undertaking involving a wide range of experiments investigating different aspects of velvet removal. The experience that I have gained in completing the various experiments has been tremendous. I have learnt several new techniques ranging from behavioural observation and analysis to laboratory sample processing. I have also practiced the art of experiment failure, improvement and improvisation and attempted to draw meaningful results from experiments where samples have been damaged or lost. Although, the statistical analyses used in this thesis have been fairly simple, a greater understanding and appreciation of statistics has been another personal outcome for me. Completing the experiments in this thesis and drawing conclusions has inspired me to continue as a veterinary researcher and made me aware of some of the pitfalls and difficulties associated with a research career.

When I started the experiment in Chapter 2, I was a new graduate veterinarian with very little clinical experience. I wanted to work in large animal practice but had little confidence in working with farmers. Going straight out onto farms and working with deer farmers that were welcoming, open minded and enthusiastic was a great start to a rural vet's career and has inspired me to focus my research future on working with the farming sector in New Zealand where I hope to be able to develop solutions to some farming challenges or improvements to current techniques.

References

- Abeyesinghe SM and Goddard PJ.** The preferences and behaviour of farmed red deer (*Cervus elaphus*) in the presence of other farmed species. *Applied Animal Behaviour Science* 56(1), 59-69, 1998
- Adams JL.** Innervation and blood supply of the antler pedicle of the red deer. *New Zealand Veterinary Journal* 27, 200-201, 1979
- Anon.** Animals Protection Act 1960 (repealed). *Government, NZ*
- Anon.** Veterinary Surgeons Act 1966. *Acts of Parliament, Government, UK.*
- Anon.** National Toxicology Program. NTP Toxicology and Carcinogenesis Studies of 2,6-Xyldine (2,6-Dimethylaniline) (CAS No. 87-62-7) in Charles River CD Rats (Feed Studies). *National Toxicology Programme Technical Report Series* 278:1-138 1990
- Anon.** Code of Recommendations and Minimum Standards During the Removal of Antlers *Code of Animal Welfare No.5, Government, NZ* 1992
- Anon.** Code of Recommendation and Minimum Standards for the Welfare of Animals Transported in New Zealand. *Code of Animal Welfare No. 15 Government, NZ,* 1994
- Anon.** Guidelines for the Welfare of Red and Wapiti Stags During the Use of Rubber Rings to Induce Analgesia for the Removal of Spiker Velvet. *Animal Welfare Advisory Committee* 1998
- Anon.** Animal Welfare (Painful Husbandry Procedures) Code of Welfare 2005, *Code of Welfare No.7, Government, NZ* 2005a
- Anon.** National Velveting Standards Body (NVSb) Farmer Velvet Antler Removal Programme. Deer Industry New Zealand 2005b
- Anon.** Evaluation of a new Class 1 substance: Deer Velvet Antler. Office of Complimentary Medicines and Medsafe IJEACCM Item 3.1, 2006
- Anon.** Veterinarians Act 1999 No 126, Public Act. *New Zealand Legislation: Acts Government, NZ* <http://www.legislation.govt.nz/act/public/2005/0126/latest/DLM363859.html> July 2009a
- Anon.** Welfare implications of Deer Velvet. *American Veterinary Medical Association.* June 2009b
- Anon.** Animal Welfare Act 1999 No 142 (As at 07 July 2010), Public Act. *New Zealand Legislation: Acts* Ministry of Agriculture and Forestry, Government, NZ <http://www.legislation.govt.nz/act/public/1999/0142/latest/DLM49664.html> 2010
- Anon.** Agricultural Compounds and Veterinary Medicines (ACVM) Act 1997, No 87 Public Act. *New Zealand Legislation: Acts Government, NZ* <http://www.legislation.govt.nz/act/public/1997/0087/latest/DLM414577.html> July 2011
- Anon.** Deer Industry New Zealand Annual Report 2010/11. *Deer Industry New Zealand* 2011
- Anon.** Food Act 1981 No 45, Public Act. *New Zealand Legislation: Acts, Government, NZ.* <http://www.legislation.govt.nz/act/public/1981/0045/13.0/DLM48687.html> July 2011
- Antognini JF and Carstens E.** Isoflurane blunts electroencephalographic and thalamic-reticular formation responses to noxious stimulation in goats. *Anesthesiology* 91(6), 1770-1779, 1999
- Antunes LM, Golledge HD, Roughan JV and Flecknell PA.** Comparison of electroencephalogram activity and auditory evoked responses during isoflurane and halothane anaesthesia in the rat. *Veterinary Anaesthesia and Analgesia* 30(1) 15-23, 2003a

- Antunes LM, Roughan JV and Flecknell PA.** Effects of different propofol infusion rates on EEG activity and AEP responses in rats. *Journal of Veterinary Pharmacology* 26, 369-376, 2003b
- Arnemo JM, Moe SR & Sølvi NE.** Xylazine-induced sedation in axis deer (*Axis axis*) and its reversal with atipamezole. *Veterinary Research Communications* 17(2), 123-128 1993
- Aziz MA & Carlyle SS.** Cardiovascular and Respiratory Effects of Xylazine in Sheep. *Zentralblatt für Veterinärmedizin Reihe A* 25, 173-180 1978
- Bacon PJ, Jones JG, Taylor P, Stewart S, Wilson-Nunn D & Kerr M.** Impairment of gas exchange due to alveolar oedema during xylazine sedation in sheep; absence of a free radical mediated inflammatory response. *Research in Veterinary Science* 65, 71-75 1998
- Bagonluri MT, Woodbury MR, Reid RS and Boison JO.** Analysis of Lidocaine and Its Major Metabolite, Monoethylglycinexylidide, in Elk Velvet Antler by Liquid Chromatography with UV Detection and Confirmation by Electrospray Ionization Tandem Mass Spectrometry. *Journal of Agricultural Food and Chemistry*, 53, 2386-2391, 2005
- Barnett JL.** Measuring pain in animals. *Australian Veterinary Journal* 75(12), 878-879 1997
- Bartels M, Wilson P, Caulkett N, Stafford K, Mellor D.** Dynamics of local anaesthetics for velvet antler analgesia. *Proceedings of a Deer Course for Veterinarians*. 18, Deer Branch NZVA, 100-108, 2001
- Benarroch EE.** The locus ceruleus norepinephrine system: Functional organization and potential clinical significance. *Neurology* 73, 1699-1704 2009
- Blumstein DT and Daniel JC.** Quantifying behaviour the JWatcher way. Sinauer Associates, USA, 2007
- Bouda J, Dávalos-Flores JL, Núñez-Ochoa L, Paasch-Martínez L & Quiroz-Rocha GF.** Blood acid-base and serum electrolyte values in red deer (*Cervus elaphus*). *Canadian Journal of Veterinary Research* 222-225 2000
- Bouts T, Taylor P, Berry K, Routh A & Gasthuys F.** Evaluation of medetomidine-ketamine and dexmedetomidine-ketamine in Chinese water deer (*Hydropotes inermis*). *Veterinary Anaesthesia and Analgesia* 38, 106-112 2011
- Boyes RN, Adams HJ & Duce BR.** Oral absorption and disposition kinetics of lidocaine hydrochloride in dogs. *Journal of Pharmacology and Experimental Therapeutics* 174 (1), 1-8 1970
- Briese E & Cabanac M.** Stress hyperthermia: physiological arguments that it is a fever. *Physiology and Behaviour* 49(6), 1153-1157 1991
- Brockman RP.** Effect of xylazine on plasma glucose, glucagon and insulin concentrations in sheep. *Research in Veterinary Science* 30(3), 383-384 1981
- Bueno AC, Cornick-Seahorn J, Seahorn TL, Hosgood G & Moore RM.** Cardiopulmonary and sedative effects of intravenous administration of low doses of medetomidine and xylazine to adult horses. *American Journal of Veterinary Research* 60(11), 1371-1376 1999
- Buerkle H & Yaksh TL.** Pharmacological evidence of different alpha 2-adrenergic receptor sites mediating analgesia and sedation in the rat. *British Journal of Anaesthesia* 81(2), 208-215 1998

- Butterworth JF & Strinchartz GR.** The alpha 2-adrenergic agonists clonidine and guanfacine produce tonic and phasic block of conduction in rat sciatic nerve fibres. *Anesthesia and Analgesia* 76, 295-301 1993
- Campbell AC.** Implementation of the velvet harvesting code of conduct. *Proceedings of a Deer Course for Veterinarians* 10, Deer Branch NZVA, 17-22, 1993
- Campbell AC.** Future Issues for Velvet Harvesting. *Proceedings of a Deer Course for Veterinarians* 11, Deer Branch NZVA, 38-41, 1994
- Campbell, AC.** Deer sedation, Prescription Animal Remedies and Veterinarians' Liability. *Proceedings of a Deer Course for Veterinarians, No.18*, Deer Branch of the New Zealand Veterinary Association: 113-114. 2001
- Campbell KB, Klavano PH, Richardson P & Alexander JE.** Hemodynamic effects of xylazine in the calf. *American Journal of Veterinary Research* 40, 1777-11780 1979
- Caron JP & LeBlanc PH.** Caudal epidural analgesia in cattle using xylazine. *Canadian Journal of Veterinary Research* 53(4), 486-489 1989
- Carrasco L, Gómez-Villamandos, Bautista MJ, Hervás J, Pulido B and Sierra MA.** Pulmonary intravascular macrophages in deer. *Veterinary Research* 27, 71-77, 1996
- Carson BL.** Local anaesthetics that metabolise to 2,6-Xylidine or *o*-Toluidine, Final Review of Toxicological literature, Integrated laboratory Systems, Research Triangle Park, North Carolina. 2000
- Cartmell SM, Mitchell D** Attenuation of reperfusion hyperalgesia in the rat by systemic administration of benzodiazepines. *British Journal of Pharmacology* 110(3) 1067-1072 1993
- Caulkett N, Cribb PH & Duke T.** Xylazine epidural analgesia for cesarean section in cattle. *Canadian Veterinary Journal* 34(11), 674-676 1993
- Caulkett NA, Cribb PH & Haigh JC.** Comparative cardiopulmonary effects of carfentanil-xylazine and medetomidine-ketamine used for immobilisation of mule deer and mule deer/white-tailed deer hybrids. *Canadian Journal of Veterinary Research* 64, 64-68 2000
- Caulkett N and Haigh JC.** Anaesthesia of North American Deer. In Zoological Restraint and Anesthesia D Heard (Ed) International Veterinary Information Services (www.ivis.org), Ithaca, New York, USA. 2 April, 2004
- Celly CS, Atwal OS, McDonell WN and Black WD.** Histopathological alterations induced in the lungs of sheep by use of α_2 -adrenergic receptor agonists. *American Journal of Veterinary Research*, 60 154-161. 1999a
- Celly CS, McDonell WN and Black WD.** Cardiopulmonary Effects of the α_2 -Adrenoceptor agonists Medetomidine and ST-91 in Anesthetised Sheep. *Journal of Pharmacology and Experimental Therapeutics* 289 (2) 712-220, 1999b
- Celly CS, McDonell WN, Young SS and Black WD.** The comparative hypoxaemic effects of four α_2 adrenoceptor agonists (xylazine, romifidine, detomidine and medetomidine) in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, 20 464-471, 1997
- Charlton JE.** *Core Curriculum for Professional Education in Pain*. 3rd edition. Seattle, IASP press. 2005

- Clarke TP, Purohit RC & Wilson RC.** Evaluation of sedative and analgesic properties of detomidine in goats. *Agricultural Practice* 14(4), 29-33 1993
- Clear MH and Morris SM** Compliance with residue requirements in velvet antler. *Proceedings of the Deer Branch of the NZVA*, 22, 13-14. 2005
- Coderre TJ, Xanthos DN, Francis L, Bennet GJ** Chronic post-ischaemic pain (CPIP): a novel animal model of complex regional pain syndrome- Type I (CRPS-1; reflex sympathetic dystrophy) produced by prolonged hindpaw ischaemia and reperfusion in the rat. *Pain* 112(1-2) 94-105 2004
- Conway A.** An update of the national velveting standards body's activities. *Proceedings of a Deer Course for Veterinarians*. Deer Branch of the New Zealand Veterinary Association: 22 110-112. 2005
- Cook NJ, Church JS, Schaefer AL, Webster JR, Matthews LR & Suttie JM.** Stress and pain assessment of velvet antler removal from Elk (*Cervus elaphus canadensis*) and Reindeer (*Rangifer tarandus*). *Online Journal of Veterinary Research* 9(1), 24-36 2005
- Cook NJ and Schaefer AL** Stress responses of wapiti (*Cervus elaphus canadensis*) to removal of velvet antler. *Canadian Journal of Animal Science* 82, 11-17, 2002
- Correa-Sales C, Rabin BC & Maze M.** A Hypnotic Response to Dexmedetomidine, an [alpha]2 Agonist, Is Mediated in the Locus Coeruleus in Rats. *Anesthesiology* 76(6), 948-952 1992
- Cottrell DF and Molony V** Afferent activity in the superior spermatic nerve of lambs - Effects of application of rubber castration rings *Veterinary Research Communications* 19(6) 503-515 1995
- Cox SK, Hammer T & Bartges J.** Monoethylglycinexylidide and lidocaine determination in porcine microsomal preparations. *Journal of Pharmaceutical and Biomedical Analysis* 37 (4), 801-804 2005
- Coyle DE & Denson DD.** Simultaneous measurement of bupivacaine, etidocaine, lidocaine, mepericaine, mepivacaine and methadone. *Therapeutic Drug Monitoring* 8 (1), 98-101 1986
- Crews JC, Cahall M, Behbehani MM.** The neurophysiological mechanisms of tourniquet pain - the activity of neurons in the rostroventral medulla in the rat. *Anesthesiology* 81(3) 730-736 1994
- Crews JC, Sehlhorst CS** Response to maintenance of tourniquet inflation in a primate model. *Regional Anaesthesia* 16(4) 195-198, 1991
- Cross JP.** Haptoglobin and plasma viscosity as markers of acute phase reactions in red deer. *Proceedings of the Deer branch of the New Zealand Veterinary Association* 5, 111-115 1988
- Cross JP & Griffin JTF.** The acute inflammatory response in farmed red deer (*Cervus elaphus*). *Proceedings of the deer branch of the New Zealand Veterinary Association* 6, 151-157 1989
- Cross JP, Griffin JTF & Mackintosh CG.** Influence of Xylazine on Hematology Values in Farmed Red Deer. *The Biology of Deer - Proceedings of the International Symposium on the Biology of Deer* Ed RD Brown 136-140 1992
- Cross JP, Mackintosh CG & Griffin JTF.** Further observations on xylazine and haematological parameters in red deer (*Cervus elaphus*): the effect on reference values, and on splenectomised

- animals. *Proceedings of the Deer branch of the New Zealand Veterinary Association* 6, 146-150 1989
- Cross JP, Mackintosh CG & Griffin JTF.** Haematological reference values for farmed deer (*Cervus elaphus*) in New Zealand. *Comparative Haematology International* 4(2), 76-85 1994
- Custer R, Kramer L & Kennedy S.** Hematological Effects of Xylazine when used for Restraint of Bactrian Camels. *Journal of the American Veterinary Medical Association* 171(9), 899-901 1977
- Dahlin LB, Danielsen N, Ehira T, Lundborg G and Rydevik B.** Mechanical effects of compression of peripheral nerves. *Journal of Biomechanical engineering* 108(2), 120-122, 1986
- Dahlin LB, Shyu BC, Danielsen N and Andersson SA** Effects of nerve compression or ischaemia on conduction properties of myelinated and non-myelinated nerve fibres. An experimental study in the rat common peroneal nerve. *Acta Physiol Scand* 136(1), 97-105, 1989
- Dalefield RR & Oehme FW.** Deer velvet antler: Some unanswered questions of toxicology. *Veterinary and Human Toxicology* 41(1) 39-41, 1999
- Davis EB, Brakora KA & Lee AH** Evolution of ruminant headgear: a review *Proceedings of the Royal Society B* 278(1720) 2857-2865 2011
- DelGiudice GD, Seal US & Kreeger TJ.** Xylazine and ketamine -induced glycosuria in white-tailed deer. *Journal of Wildlife Diseases* 24(2), 317-321 1988
- Delvaux H, Courtois R, Breton L & Patenaude R.** Relative efficiency of succinylcholine, xylazine, and carfentanil/xylazine mixtures to immobilize free-ranging moose. *Journal of Wildlife Diseases* 35(1), 38-48 1999
- Dinniss AS, Mellor DJ, Stafford KJ, Bruce RA, Ward RN.** Acute cortisol responses of lambs to castration using a rubber ring and/or a castration clamp with or without local anaesthetic. *New Zealand Veterinary Journal* 45(3) 114-121 1997
- Dinniss AS, Stafford KJ, Mellor DJ, Bruce RA, Ward RN** The behaviour pattern of lambs after castration using a rubber ring and/or castration clamp with or without local anaesthetic. *New Zealand Veterinary Journal* 47(6) 198-203 1999
- Doherty TJ, Kattesh HG, Adcock RJ, Welborn MG, Saxton AM, Morrow JL and Dailey JW.** Effects of a Concentrated Lidocaine Solution on the Acute Phase Stress Response to Dehorning in Dairy Calves. *Journal of Dairy Science* 90 (9), 4232-4239, 2007
- Doherty TJ, Pascoe PJ, McDonell WN & Monteith G.** Cardiopulmonary effects of xylazine and yohimbine in laterally recumbent sheep. *Canadian Journal of Veterinary Research* 50(4), 517-521 1986
- DuBois WR, Prado TM, Ko JCH, Mandsager RE and Morgan GL.** A comparison of two intramuscular doses of xylazine-ketamine combination and tolazoline reversal in llamas. *Veterinary Anaesthesia and Analgesia* 31, 90-96, 2004
- Duffield TF, Heinrich A, Millman ST, DeHaan A, James S and Lissemore K.** Reduction in pain response by combined use of local lidocaine anesthesia and systemic ketoprofen in dairy calves dehorned by heat cauterization. *Canadian Veterinary Journal* 51(3), 283-288, 2010
- Duhm B, Maul W, Medenwald H, Patzschke K & Wegner LA.** Experiments using radioactively

- tagged BAY Va 1470 on rats. *Berl. Münch. Tierärztl. Wochenschr.* 82, 104-109 1969
- Dwyer RC, Rampil IJ, Eger EI and Bennet HL.** The electroencephalogram does not predict depth of isoflurane anaesthesia. *Anaesthesiology* 81, 403-409, 1994
- Eisenach JC.** Intravenous Clonidine Produces Hypoxaemia by a Peripheral Alpha-2 Adrenergic Mechanism. *Journal of Pharmacology and Experimental Therapeutics* 244, 247-252, 1988
- Eisenach JC, De Kock M & Klimscha W.** α_2 -Adrenergic Agonists for Regional Anesthesia: A Review of Clonidine (1984-1995). *Anesthesiology* 85(3), 655-674 1996
- Eichner RD, Prior RL & Kvasnicka WG.** Xylazine-induced hyperglycemia in beef cattle. *American Journal of Veterinary Research* 40(1), 127-129 1979
- Faulkner PM and Weary DM.** Reducing pain after dehorning in dairy calves. *Journal of dairy science* 83(9) 2037-2041, 2000
- Fayed AH, Abdalla EB, Anderson RR, Spencer K and Johnson HD.** Effect of xylazine in heifers under thermoneutral and heat stress conditions. *American Journal of Veterinary Research* 50(1), 151-153, 1989
- Fern R and Harrison PJ.** The contribution of ischaemia and deformation to the conduction block generated by compression of the cat sciatic nerve. *Experimental Physiology* 79(4), 583-92, 1994a
- Fern R and Harrison PJ.** The relationship between ischaemic conduction failure and conduction velocity in cat myelinated axons. *Experimental Physiology* 79(4), 571-81, 1994b
- Fernandández-Morán J, Palomeque J & Peinado VI.** Medetomidine/tiletamine/zolazepam and xylazine/tiletamine/zolazepam combination for immobilization of fallow deer (*Cervus dama*). *Journal of Zoo and Wildlife Medicine* 31(1), 62-64 2000
- Fletcher J.** Hypersensitivity of an isolated population of red deer (*Cervus elaphus*) to xylazine. *Veterinary Record* 94(5), 85-86 1974
- Firn S.** Accidental poisoning by animal immobilising agent. *Lancet* (ii), 95 1973
- Fluorescent Microsphere Resource Centre, University of Washington, Seattle, WA, <http://fmrc.pulmcc.washington.edu>
- Forrest JA, Finlayson ND, Adjepon-Yamoah KK & Prescott LF.** Antipyrine, paracetamol, and lignocaine elimination in chronic liver disease. *British Medical Journal* 28 (1(6073)), 1384-1387 1977
- Fox JL and Kenmore PI.** The Effect of Ischemia on Nerve Conduction. *Experimental Neurology* 17, 403-419, 1967
- Fukuda T, Kakiuchi Y, Miyabe M, Kihara S, Kohda Y & Toyooka H.** Free lidocaine concentrations during continuous epidural anaesthesia in geriatric patients. *Regional Anaesthesia and Pain Medicine* 28 (3), 215-220 2003
- Gan J, Skipper PL, Manuela G-D, Arakawa K, Ross RK, Yu MC & Tannenbaum SR.** Alkyl-aniline-Hemoglobin Adducts and Risk of NonSmoking-Related Bladder Cancer. *Journal of the National Cancer Institute* 96(19), 1425-1431 2004
- Garcia-Villar R, Toutain PL, Alvinerie M & Ruckebusch Y.** The pharmacokinetics of xylazine

- hydrochloride: an interspecific study. *Journal of Veterinary Pharmacology and Therapeutics* 4, 87-92 1981
- Gelgor L, Cartmell S, Mitchell D** Intracerebroventricular micro-injections of nonsteroidal anti-inflammatory drugs abolish reperfusion hyperalgesia in the rats tail. *Pain* 50(3) 323-329 1992
- Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL & Mellor DJ.** Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal* 55 (4), 152-157 2007
- Glenny RW, Bernard S, Brinkley M.** Validation of fluorescent labelled microspheres for measurement of regional organ perfusion. *Journal of Applied Physiology* 74(5): 2585-2597, 1993
- Goodrich PGE.** Accidental self-injection. *Veterinary Record* 100, 458-459 1977
- Gordh T, Gordh TE & Lindqvist K.** Lidocaine: The origin of a modern local anaesthetic. *Anaesthesiology* 113, 1433-1437 2010
- Gorewit RC.** Effects of clonidine on glucose production and insulin secretion of cattle. *American Journal of Veterinary Research* 41(11), 1769-1772 1980
- Goss RJ.** Deer Antlers: Regeneration, Function, and Evolution (Academic Press Inc, London, 1983).
- Goss RJ & Powel RS.** Induction of deer antlers by transplanted periosteum. I. Graft size and shape. *Journal of Experimental Zoology* 235(3), 359-373 1985
- Goss RJ, Van Praagh A & Brewer P.** The mechanism of antler casting in fallow deer. *Journal of Experimental Zoology* 264(4), 429-436 1992
- Graf B and Senn M.** Behavioural and physiological responses of calves to dehorning by heat cauterization with or without local anaesthesia. *Applied Animal Behaviour Science* 62(1-2) 153-171, 1999
- Graham MJ, Kent JE and Molony V** Effects of four analgesic treatments on the behavioural and cortisol responses of 3-week-old lambs to tail docking *Veterinary Journal* 153(1) 87-97 1997
- Greene SA & Thurmon JC.** Xylazine - a review of its pharmacology and use in veterinary medicine. *Journal of Veterinary Pharmacology and Therapeutics* 11, 295-313 1988
- Greene S, Thurmon JC, Tranquilli WJ & Benson GJ.** Effect of yohimbine on xylazine-induced hypoinsulinemia and hyperglycemia in mares. *American Journal of Veterinary Research* 48(4), 676-678 1987
- Gross ME & Tranquilli WJ.** Use of Alpha 2-Adrenergic Receptor Antagonists. *Journal of the American Veterinary Medical Association* 195(3), 378-381 1989
- Guard CL & Schwark WS.** Influence of yohimbine on xylazine-induced depression of central nervous, gastrointestinal and cardiovascular function in the calf. *Cornell Vet* 74(4), 312-321 1984
- Haas LF.** Hans Berger (1873-1941), Richard Caton (1842-1926) and electroencephalography. *Journal of neurology, neurosurgery and psychiatry* 74(1) 9, 2003
- Haigh JC & Caulkett N.** New and not so new drugs or restraint and sedation in deer. *Proceedings of the deer branch of the New Zealand Veterinary Association* 157-161 2004

- Haigh JC & Hudson RJ.** Farming of wapiti and red deer. St. Louis, Mosby-Year Book, Inc 83-98 1993
- Haigh JC, Woodbury MR, Stokey JM, Schaefer AL, Caulkett NA & Wilson PR** New Technologies for velvet antler removal: The search for the ideal method. In: *Antler Science and Product Technology* Sim JS Sunwoo HH Hudson RJ and Jeon BT (eds.) ASPTRC, Edmonton, 387-409, 2001
- Haines SR & Suttie JM.** DeerVelvet Technical manual *Deer Industry New Zealand* Version 6, 2008
- Haskins SC, Patz JD & Farver TB.** Xylazine and xylazine-ketamine in dogs. *American Journal of Veterinary Research* 47(3), 636-641 1986
- Haskins SC, Peiffer RLJ & Stowe CM.** A clinical comparison of CT1341, ketamine and xylazine in cats. *American Journal of Veterinary Research* 36(10), 1537-1543 1975
- Hefti E, Trechsel U, Rüfenacht H and Fleisch H.** Use of dermestid beetles for cleaning bones. *Calcified Tissue International* 31(1), 45-47, 1980
- Hoffman PE.** Clinical evaluation of xylazine as a chemical restraining agent, sedative and analgesic in horses. *Journal of the American Veterinary Medical Association* 164, 42-45 1974
- Hoffmann AC, vanGessel E, Gamulin Z, Ryser JE ad Forster A.** Quantitative evaluation of tourniquet leak during i.v. regional anaesthesia of upper and lower limbs in human volunteers. *British journal of Anaesthesia* 75 (3) 269-273, 1995
- Hoffmann R.** Country of origin - a consumer perceptive perspective of fresh meat. *British Food Journal* 102(3) 211-229 2000
- Honkavaara JM, Restitutti F, Raekallio MR, Kuusela EK & Vainio OM.** The effects of increasing doses of MK-467, a peripheral $\alpha(2)$ -adrenergic receptor antagonist, on cardiopulmonary effects of intravenous dexmedetomidine in conscious dogs. *Journal of Veterinary Pharmacology and Therapeutics* 34(4), 332-337 2011
- Hsu WH.** Yohimbine increases plasma insulin concentrations and reverses xylazine-induced hypoinsulinemia in dogs. *American Journal of Veterinary Research* 49(2), 242-244 1988
- Hsu WH, Lu ZX & Hembrough FB.** Effect of xylazine on heart rate and arterial blood pressure in conscious dogs, as influenced by atropine, 4-aminopyridine, doxapram, and yohimbine. *Journal of the American Veterinary Medical Association* 186(2), 153-156 1985
- Hsu WH, Hanson CE, Hembrough FB and Schaffer DD.** Effects of idazoxan, tolazoline, and yohimbine on xylazine induced respiratory changes and central nervous system depression in ewes. *American Journal of Veterinary Research* 50 (9), 1570-1573, 1989
- IARC (International Agency for Research on Cancer)** IARC monographs on the Evaluation of the Carcinogenic Risks of Chemicals to Humans. Vol 57, 2,6-dimethylaniline (2,6-xylidine) 323-335, IARC Press, Lyons, France.
- Irvine WJ & Saxby MJ.** Steam volatile amines of Latakia tobacco leaf. *Phytochemistry* 8, 473-476 1979
- Janicki Z, Konjević D, Slavica A & Severin K.** Reversible chemical immobilization of wild red deer (*Cervus elaphus* L.) using tiletamine-zolazepam-xylazine hydrochloride mixture. *Veteri-*

- narski Arhiv* 76(3), 237-244 2006
- Janovsky M, Tataruch F, Ambuehl M and Giacometti M.** A zoletil®-Rompun® mixture as an alternative to the use of opioids for immobilisation of feral red deer. *Journal of Wildlife Diseases* 36(4) 663-669, 2000
- Janssen PAJ.** The evolution of butyrophenones, haloperidol, and trifluoperidol, from meperidine-like 4-phenylpiperidines. *International Review of Neurobiology* 8, 221-263 1965
- Johansen O.** Comparison of articaine and lidocaine used as dental local anaesthetics. Section of Dental Pharmacology and Pharmacotherapy, Institute of Clinical Dentistry, Faculty of Dentistry Project Thesis (H-99), 2004
- Johnson C.** Research methods for pain evaluation. *Proceedings of a Deer Course for Veterinarians* 20, 63-67, 2003
- Johnson CB, Bloomfield M and Taylor PM.** Effects of ketamine on the equine electroencephalogram during anesthesia with halothane in oxygen. *Veterinary Surgery* 28(5), 380-385, 1999
- Johnson CB and Taylor PM.** Comparison of the effects of halothane, isoflurane and methoxyflurane on the electroencephalogram of the horse. *British Journal of Anaesthesia* 81, 748-753, 1998
- Johnson CB, Wilson PR, Woodbury MR, Caulkett NA.** Comparison of analgesic techniques for antler removal in halothane-anaesthetised red deer (*Cervus elaphus*): electroencephalographic responses. *Veterinary Anaesthesia and Analgesia*, 32, 61-71, 2005
- Johnson C, Woodbury, M., Caulkett, N. & Wilson, PR.** Comparison of lidocaine and antler compression for analgesia for Velvet Antler Removal in Red Deer (*Cervus elaphus*) Anaesthetised by Halothane in Oxygen. *Proceedings of a Deer Course for Veterinarians* 20, Deer Branch NZVA, 79-90, 2003
- Jongman EC, Morris JP, Barness JL, Hemsworth PH.** EEG changes in 4-week-old lambs in response to castration, tail docking and mulesling. *Australian Veterinary Journal* 78(5) 339-343, 2000
- Joyce C, Smyth WF, Ramachandran VN, O'Kane E & Coulter DJ.** The characterisation of selected drugs with amine-containing side chains using electrospray ionisation and ion trap mass spectrometry and their determination by HPLC-ESI-MS. *Journal of Pharmaceutical and Biomedical Analysis* 36, 465-476 2004
- Kent JE, Molony V and Robertson IS** Comparison of the burdizzo and rubber ring methods for castration and tail docking lambs. *Veterinary Record* 136(8) 192-196 1995
- Kent JE, Thrusfield MV, Molony V, Hosie BD & Sheppard BW.** Randomised, controlled field trial of two new techniques for the castration and tail docking of lambs less than two years of age. *Veterinary Record* 154(7), 193-200 2004
- Keenaghan JB and Boyes RN.** The tissue distribution, metabolism and excretion of lidocaine in rats, guinea pigs, dogs and man. *The Journal of Pharmacology and experimental Therapeutics*, 180(2), 454-463, 1972
- Kimura J.** Principles and pitfalls of nerve conduction studies. *Annals of Neurology* 16(4) 415-429 1984
- Klide AM, Calderwood HW & Simen LR.** Cardio-pulmonary effects of xylazine in dogs. *Ameri-*

- can Journal of Veterinary Research* 36(7), 931-935 1975
- Ko JC & McGrath CJ.** Effect of Atipamizole and Yohimbine on Medetomidine Induced Central Nervous System Depression and Cardiorespiratory Changes in Lambs. *American Journal of Veterinary Research* 56, 629-632 1995
- Kornreich M and Montgomery CA Jr.** Toxicology and carcinogenesis studies of 2,6-xylidine (2,6-dimethylaniline) in Charles River CD Rats. National Toxicology Program (NTP). NTP TR 278, NIH Publication number 90-2534 1-138, 1990
- Kreeger TJ, Huizenga M, Hansen C & Wise BL.** Sufentanil and xylazine immobilisation of Rocky Mountain Elk. *Journal of Wildlife Diseases* 47(3), 638-642 2011
- Kullmann A.** Effects of xylazine, romifidine and detomidine on haematology, serum biochemistry and splenic size in horses. Masters thesis, University of Pretoria 2011
- Kumar K & Holden WE.** Drug-Induced Pulmonary Vascular Disease - Mechanisms and Clinical Patterns. *Western Journal of Medicine* 145, 343-349 1986
- Lambooya E.** Electroanaesthesia or electroimmobilisation of calves, sheep and pigs by the Feenix Stockstill. *Veterinary Quarterly* 7(2), 120-126 1985
- Leduc ML, Atherley R, Jinks SL and Antognini JF.** Nitrous oxide depresses electroencephalographic responses to repetitive noxious stimulation in the rat. *British Journal of Anaesthesia* 96(2), 216-221, 2006
- Lester SJ, Mellor DJ, Ward RN, Holmes RJ** Cortisol responses of young lambs to castration and tailing using different methods. *New Zealand Veterinary Journal* 39(4) 134-138 1991
- Li C, Sheard PW, Corsen ID and Suttie JM.** Pedicle and antler development following sectioning of the sensory nerves to the antlerogenic region of red deer (*Cervus elaphus*). *Journal of Experimental Zoology* 267 (2), 131-38, 1993
- Li C & Suttie JM.** Light Microscopic Studies of Pedicle and Early First Antler Development in Red Deer (*Cervus elaphus*). *The Anatomical Record* 239, 198-215 1994
- Lin HC, Thurmon JC, Benson GJ, Tranquilli WJ and Olson WA.** The Hemodynamic response of Calves to Tiletamine-Zolazepam Anesthesia. *Veterinary Surgery* 18(4), 328-334, 1989
- Lin X, Yang H, Sakuragi T, Hu M, Mantell LL, Hayashi S, Al-Abed Y, Tracey KJ and Miller EJ.** -Chemokine receptor blockade reduces high mobility group box 1 protein - induced lung inflammation and injury and improves survival in sepsis. *American Journal of Physiology Lung Cell Molecular Physiology* 289 L583-L590, 2005
- Longworth KE, Smith BL, Staub NC, Steffey EP and Serikov VB.** Use of detergent to prevent initial responses to endotoxin in horses. *Australian Journal of Veterinary Research* 7, 1063-1066, 1996
- Lorec AM, Bruguerolle B, Attolini L & Roucoules X.** Rapid simultaneous determination of lidocaine, bupivacaine, and their two main metabolites using capillary gas-liquid chromatography with nitrogen-phosphorus detector. *Therapeutic Drug Monitoring* 16 (6), 592-595 1994
- McCorquodale SM, Eberhardt LE & Petron SE.** Helicopter Immobilization of Elk in South-central Washington. *Northwest Science* 62(1), 49-52 1988

- McCracken L, Waran N, Mitchinson S and Johnson CB.** Effect of age at castration on behavioural response to subsequent tail-docking in lambs. *Veterinary Anaesthesia and Analgesia* 37(4), 375-381, 2010
- Machado HS & Bastos RS.** Inadequate tourniquet inflation associated with a case of prilocaine toxicity. *European Journal of Anaesthesiology* 15, 234-236 1998
- Mackintosh CG.** Immobilon/Revivon in Red Deer. *Proceedings of the Deer branch of the New Zealand Veterinary Association* 2, 10-19 1985
- Mackintosh CG and Cross JP.** Xylazine study report. *Proceedings of a Deer Course for Veterinarians, No.6*, Deer Branch of the New Zealand Veterinary Association: 136-143. 1989
- Mackintosh CG and Van Reenen G.** Comparison of yohimbine, 4-aminopyridine and doxapram antagonism of xylazine sedation in deer (*Cervus elephus*). *New Zealand Veterinary Journal* 32 181-184, 1984
- McMeekan CM, Mellor DJ, Stafford KJ, Bruce RA, Ward RN & Gregory NG.** Effects of local anaesthesia of 4 and 8 hours' duration on the acute cortisol response to scoop dehorning in calves. *Australian Veterinary Journal* 76(4), 281-285 1998
- McMeekan CM, Stafford KJ, Mellor DJ, Bruce RA, Ward RN and Gregory N.** Effects of a local anaesthetic and a non-steroidal anti-inflammatory analgesic on the behavioural responses of calves to dehorning. *New Zealand Veterinary Journal* 47(3) 92-96, 1999
- McNeel SV & Hsu WH.** Xylazine-induced prolongation of gastrointestinal transit in dogs: reversal by yohimbine and potentiation by doxapram. *Journal of the American Veterinary Medical Association* 185(8), 878-881 1984
- Main DCJ, Waterman AE, Kilpatrick IC, Jones A.** An assessment of the peripheral antinociceptive potential of remoxipride, clonidine and fentanyl in sheep using the forelimb tourniquet. *Journal of Veterinary Pharmacology and Therapeutics* 20(3) 220-228 1997
- Malony V, Kent JE and Robertson IS.** Behavioural responses of lambs of three ages in the first three hours after three methods of castration and tail docking. *Research in Veterinary Science* 55(2), 236-245, 1993
- Manning MM and Broadstone RV.** Effects of α_2 -adrenergic receptor agonist and antagonist drugs on cholinergic contraction in bovine tracheal smooth muscle *in vitro*. *American Journal of Veterinary Research*, 56 (7), 930-935, 1995
- Marco I & Lavin S.** Effect of the method of capture on the haematology and blood chemistry of red deer (*Cervus elaphus*). *Research in Veterinary Science* 66(2), 81-84 1999
- Marshall BL.** Chemical residues and venison exports. *Proceedings of the deer branch of the New Zealand Veterinary Association*. 8, 44-51 1991
- Matthews LR, Bremner KJ, Pearse AJT, Morrow CJ and Webster JR.** Evaluation of compression analgesia for velvet removal in red deer. *Journal of Animal Science*, 80, 372, Abstract 1491, 2002
- Matthews LR, Carragher JF and Ingram JR.** Post-velveting stress in free-ranging red deer. *Proceedings of the deer branch of the New Zealand Veterinary Association*. 11, 138-146, 1994
- Matthews LR, Cook C and Asher GW.** Behavioural and physiological responses to management

- practices in red deer stags. *Proceedings of a deer course for veterinarians* 7, 74-85, 1990
- Matthews LR and Cook CJ** Deer Welfare Research - Ruakura findings. *Proceedings of a deer course for veterinarians* 8. *Deer branch of the NZVA* 120-127, 1991
- Matthews LR, Ingram J, Cook C, Bremner K, Kirton P.** Induction and assessment of velvet analgesia. *Proceedings of a Deer Course for Veterinarians* 9, Deer Branch NZVA, 69-76, 1992
- Matthews LR Pollard J Ingram J Mackintosh C Suttie J Morrow C and Bremner K** Non-chemical techniques for inducing analgesia prior to velvetting: I. Electronic analgesia. *Proceedings of a Deer Course for Veterinarians* 16, 189-191, 1999a
- Matthews LR, Bremner KJ and Morrow CJ.** Non-chemical techniques for inducing analgesia prior to velvetting: II The effect of the NaturO™ technique on subsequent antler production. *Proceedings of a Deer Course for Veterinarians* 16, 193-194, 1999b
- Matthews LR and Suttie JM.** Research Progress in Non-Chemical Techniques for Inducing Analgesia Prior to Velvet Antler Removal. In *Antler Science and Product Technology* Eds. Sim JS, Sunwoo HH, Hudson RJ and Jeon BT. 411-425, 2001
- Mautz WW, Seal US & Boardman CB.** Blood Serum Analyses of Chemically and Physically Restrained White-Tailed Deer. *The Journal of Wildlife Management* 44(2), 343-351 1980
- Mellor DJ & Stafford KJ.** Interpretation of cortisol responses in calf disbudding studies. *New Zealand Veterinary Journal* 45, 126-127 1997
- Mellor DJ & Stafford KJ.** Acute castration and/or tailing distress and its alleviation in lambs. *New Zealand Veterinary Journal* 48, 33-43 2000
- Melzack R.** From the gate to the neuromatrix. *Pain* 82(supplement 1), S121-S126 1999
- Melzack R & Wall PD.** Pain Mechanisms: A New Theory. *Science, New Series* 150(3699), 971-979 1965
- Mich PM, Wolfe LL, Sirochman TM, Sirochman MA, Davis TR, Lance WR & Miller MW.** Evaluation of Intramuscular Butorphanol, Azaperone, and Medetomidine and Nasal Oxygen Insufflation for the Chemical Immobilisation of White-tailed Deer, *Odocoileus virginianus*. *Journal of Zoo and Wildlife Medicine* 39(3), 480-487 2008
- Miller BF, Muller LI, Storms TN, Ramsay EC, Osborn DA, Warren RJ, Miller KV & Adams KA.** A comparison of carfentanil/xylazine and Telazol/xylazine for immobilization of white-tailed deer. *Journal of Wildlife Diseases* 39(4), 851-858 2003
- Miller EJ, Cohen AB, Nagao S, Griffin D, Maunder RJ, Martin TR, Weiner-Kronish JP, Sticherling M, Christophers E & Matthay MA.** Elevated levels of NAP-1-interleukin-8 are present in the airspaces of patients with adult respiratory distress syndrome and are associated with increased mortality. *American Review of Respiratory Disease* 146(2), 427-432 1992
- Milligan BN, Duffield T and Lissemore K.** The utility of ketoprofen for alleviating pain following dehorning in young dairy calves. *Canadian Veterinary Journal* 45(2), 140-143, 2004.
- Millspaugh JJ, Brundige GC, Jenks JA, Tyner CL & Hustead DR.** Immobilization of Rocky Mountain elk with Telazol and xylazine hydrochloride, and antagonism by yohimbine hydrochloride. *Journal of Wildlife Diseases* 31(2), 259-262 1995

- Mitragotri S** Current status and future prospects of needle-free liquid jet injectors *Nature* 5 (July), 543-548, 2006
- Molony V, Kent JE, McKendrick IJ** Validation of a method for assessment of an acute pain in lambs. *Applied Animal Behaviour Science* 76(3) 215-238 2002
- Molony V, Kent JE, Robertson IS** Behavioural responses of lambs of 3 ages in the first three hours after 3 methods of castration and tail docking. *Research in Veterinary Science* 55(2) 236-245 1993
- Molony V, Kent JE, Robertson IS** Assessment of acute and chronic pain after different methods of castration of calves. *Applied Animal Behaviour Science* 46(1-2) 33-48 1995
- Moresco A, Larsen RS, Sleeman JM, Wild MA & Gaynor JS.** Use of naloxone to reverse car-fentanil citrate- induced hypoxaemia and cardiopulmonary depression in Rocky Mountain Wapiti (*Cervus elaphus nelsoni*). *Journal of Zoo and Wildlife Medicine* 32(1), 81-89 2001
- Morisse JP, Cotte JP and Huonnic D.** Effect of dehorning on behaviour and plasma cortisol responses in young calves. *Applied Animal Behaviour Science* 43(4), 239-247, 1995
- Morris JP, Ong RM, Barnett JL, Hemsworth PH, Clarke IJ and Jongman EC.** Pain-related cerebral potentials in response to acute painful electrical stimulation in sheep. *Australian Veterinary Journal* 75(12), 883-886, 1997
- Morrow CJ & Matthews LR.** Determining best practice for inducing analgesia in Red Deer prior to velveting by non-veterinarian operators. Report to MAF policy - confidential report. ABWRC AgResearch Ruakura. 1998
- Muggaberg J & Brockman RP.** Effect of adrenergic drugs on glucose and plasma glucagon and insulin responses to xylazine in sheep. *Research in Veterinary Science* 33(1), 118-120 1982
- Murakami T.** Pliable methacrylate casts of blood vessels: Use in scanning electron microscope study of the microcirculation in rat hypophysis. *Archivum Histologicum Japonicum* 38(2), 151-168, 1975
- Murphy JJ & Jacobs K.** Residues of ROMPUN and its metabolites in cattle. *Bayer Study Report* No. 43814, Bayer AG Leverkusen, Germany 1975
- Murray S, Monfort SL, Ware L, McShea WJ and Bush M.** Anesthesia in female white-tailed deer using telazol[®] and xylazine. *Journal of wildlife diseases* 36 (4) 670-675, 2000
- Murrell JC and Johnson CB.** Neurophysiological techniques to assess pain in animals. *Journal of Veterinary Pharmacology and Therapeutics*, 29, 325-335, 2006
- Murrell JC, Johnson CB Whitez KL, Taylor PM, Zainal L Haberham ZL and Waterman-Pearson AE.** Changes in the EEG during castration in horses and ponies anaesthetized with halothane. *Veterinary Anaesthesia and Analgesia* 30, 138-146, 2003
- Murrell JC, Mitchinson SL, Waters D and Johnson CB** Comparative effect of thermal, mechanical, and electrical noxious stimuli on the electroencephalogram of the rat. *British Journal of Anaesthesia* 98(3), 366-71, 2007a
- Murrell JC, Mitchinson SL, Johnston AC, Johnson CB & Barnes RG.** Is it possible to generate cerebral evoked potentials with a mechanical stimulus from the duodenum in rats? *Journal of Neuroscience Methods* 162, 215-221 2007b

- Murrell JC, Waters D and Johnson CB.** Comparative effects of halothane, isoflurane, sevoflurane and desflurane on the electroencephalogram of the rat. *Laboratory Animals* 42(2), 161-170, 2008
- Murrell J, White KL, Johnson CB, Taylor PM, Doherty TJ & Waterman-Pearson AE.** Investigation of the EEG effects of intravenous lidocaine during halothane anaesthesia in ponies. *Veterinary Anaesthesia and Analgesia* 32, 212-221 2005
- Mutlib AE, Chui YC, Young LM & Abbott FS.** Characterization of metabolites of xylazine produced in vivo and *in vitro* by LC/MS/MS and GC/MS. *Drug Metabolism and Distribution* 20(6), 840-848 1992
- Nelson SD, Garland WA, Breck GD & Trager WF.** Quantification of lidocaine and several metabolites utilizing chemical-ionisation mass spectrometry and stable isotope labelling. *Journal of Pharmacological Science* 66 (8), 1180-1190 1977
- Nicol AM, Barrell GK, Gibbs SJ, Frizzell AN & McPhee JF.** Assessment of the production of analgesia induced by application of a rubber ring or local anaesthetic to the antler pedicle of yearling stags. *New Zealand Veterinary Journal* 57(3), 153-159 2009
- Nitz AJ and Matulionis DH** Ultrastructural changes in rat peripheral nerve following pneumatic tourniquet compression. *Journal of Neurosurgery* 57(5), 660-666, 1982
- Nolan AM, Callingham BA & Evans RJ.** Effects of aspirin on xylazine-induced hypoxaemia in sheep. *Research in Veterinary Science* 48(3), 386-388 1990
- Nolan A, Livingstone A, Waterman A.** The effects of alpha2 adrenoceptor agonists on airway pressure in anaesthetized sheep. *Journal of Veterinary Pharmacology and Therapy* 9 157-163 1986
- Oertel R, Rahn R & Kirch W.** Clinical pharmacokinetics of articaine. *Clinical pharmacokinetics* 33(6), 417-425 1998
- Oka T, Oka K & Hori T.** Mechanisms and mediators of psychological stress-induced rise in core temperature. *Psychosomatic Medicine* 63(3), 476-486 2001
- Ormerod W.** Richard Caton (1842-1926): pioneer electrophysiologist and cardiologist. *Journal of Medical Biography* 14(1) 30-35 2006
- Orr CM.** Accidental self-injection. *Veterinary Record* 100, 574 1977
- Orth M, Barter L, Dominguez C, Atherley R, Carstens E and Angognini JF.** Halothane and propofol differentially affect electroencephalographic responses to noxious stimulation. *British Journal of Anaesthesia* 95(4), 447-484, 2005
- Orth M, Bravo E, Barter L, Carstens E, and Antognini JF.** The differential effects of halothane and isoflurane on electroencephalographic responses to electrical microstimulation of the reticular formation. *Anesthesia and Analgesia* 102(6), 1709-1714, 2006
- Otto KA & Short CE.** Pharmaceutical control of pain in large animal. *Applied Animal Behavioural Science* 59, 157-169 1998
- Parbhakar OP, Duke T, Townsend HGG and Singh B.** Depletion of pulmonary intravascular macrophages partially inhibits lipopolysaccharide-induced lung inflammation in horses. *Veterinary Research* 36, 557-569, 2005

- Parker JBR & Haigh JC** Human exposure to immobilizing agents in *Chemical Immobilization of North American Wildlife* (eds Neilson L, Haigh JC & Fowler ME) 119-136 Winsconsin Humane Society, Milwaukee, 1982
- Paterson JM, Caulkett NA & Woodbury MR.** Physiological Effects of Nasal Oxygen or Medical Air Administered Prior to and During Carfentanil-Xylazine Anesthesia in North American Elk (*Cervus canadensis manitobensis*). *Journal of Zoo and Wildlife Medicine* 40(1), 39-50 2009
- Pearce PC & Kock RA.** Physiological effects of etorphine, acepromazine and xylazine on black fallow deer (*Dama dama*). *Research in Veterinary Science* 46(3), 380-386 1989
- Petrie NJ, Mellor DJ, Stafford KJ, Bruce RA & Ward RN.** Cortisol responses of calves to two methods of disbudding used with or without local anaesthetic. *New Zealand Veterinary Journal* 44, 9-14 1995
- Pippi NL & Lumb WV.** Objective tests of analgesic drugs in ponies. *Journal of the American Veterinary Medical Association* 44, 1667-1669 1979
- Pollard J, Littlejohn R, Johnstone P, Laas F, Corson I, Suttie J.** Behavioural and heart rate responses to velvet removal in red deer. *New Zealand Veterinary Journal* 40, 56-61, 1992a
- Pollard JC, Littlejohn RP, Waldrup KA, Suttie JM** The effects of post-treatment analgesia on behavioural responses to antler removal in red deer. *Proceedings of a deer course for veterinarians* 9 49-53 1992b
- Pollard JC, Littlejohn RP & Suttie JM.** Responses of red deer to restraint in a y-maze preference test. *Applied Animal Behaviour Science* 39(1), 63-71 1994
- Pollard JC, Suttie JM, Littlejohn RP, Johnstone P, Laas FJ and Corson ID.** Measurement of behaviour and heart rate to assess the aversiveness of handling treatments used for red deer. *Proceedings of a deer course for veterinarians*, 8, 109-119, 1991
- Price JC, Allen S, Faucheux C, Althnaian T & Mount JG.** Deer antlers: a zoological curiosity or the key to understanding organ regeneration in mammals? *Journal of Anatomy* 207, 603-618 2005
- Price JS, Oyajobi BO, Nalin AM, Frazer A, Russell RG & Sandell LJ.** Chondrogenesis in the regenerating antler tip in red deer: expression of collagen types I, IIA, IIB, and X demonstrated by in situ nucleic acid hybridization and immunocytochemistry. *Developmental Dynamics* 205(3), 332-347 1996
- Prinzen FW & Glenny RW.** Developments in non-radioactive microsphere techniques for blood flow measurement. *Cardiovascular Research* 28, 1467-1475 1994
- Pusateri FM, Hibler CP & Pojar TM.** Oral administration of diazepam and promazine hydrochloride to immobilise pronghorn. *Journal of Wildlife Diseases* 18(1), 9-16 1982
- Putter J & Sagner G.** Chemical studies to detect residues of xylazine hydrochloride. *Veterinary Medical Review* 2, 145-159 1973
- Quinton DN, Hughes J, Mace PF and Aitkenhead AR.** Prilocaine leakage during tourniquet inflation in intravenous regional anaesthesia: the influence of fracture manipulation. *Injury* 19(5) 333-335, 1988
- Ranheim B, Soli NE, Ryeng KA, Arnemo JM and Horsberg TE.** Pharmacokinetics of medeto-

- midine and atipamezole in dairy calves: an agonist-antagonist interaction. *Journal of Veterinary Pharmacology and Therapeutics* 21, 428-432, 1998
- Raptopoulos D and Weaver BMQ.** Observations following intravenous xylazine administration in steers. *The Veterinary Record* 114, 567-569, 1984
- Rayner V & Ewen SW.** Do the blood vessels of the antler velvet of the red deer have an adrenergic innervation? *Quarterly Journal of Experimental Physiology* 66(1), 81-86 1981
- Read MR.** A review of α_2 adrenoceptor agonists and the development of hypoxemia in domestic and wild ruminants. *Journal of Zoo and Wildlife Medicine* 34(2), 134-138, 2003
- Read M, Caulkett N and McCallister M.** Evaluation of zuclopenthixol acetate to decrease handling stress in wapiti. *Journal of Wildlife Diseases*, 36(3), 450-459, 2000
- Read MR, Caulkett NA, Symington, A and Shury TK** Treatment of hypoxemia during xylazine-tiletamine-zolazepam immobilisation of wapiti. *Canadian Veterinary Journal* 42, 861-864, 2001
- Read MR & McCorkell RB.** Use of azaperone and zuclopenthixol acetate to facilitate translocation of white-tail deer (*Odocoileus virginianus*). *Journal of Zoo and Wildlife Medicine* 33(2), 163-165 2002
- Rioja E, Kerr CL, Enouri SS & McDonnell WN.** Sedative and cardiopulmonary effects of medetomidine hydrochloride and xylazine hydrochloride and their reversal with atipamezole hydrochloride in calves. *American Journal of Veterinary Research* 69(3), 319-329 2008
- Risling TE, Fahlman A, Caulkett NA & Kutz S.** Physiological and behavioural effects of hypoxaemia in reindeer (*Rangifer tarandus*) immobilised with xylazine-etorphine. *Animal Production Science* 51(4), 355-358 2011
- Robertson SA, Carter SW, Donovan M & Steele C.** Effects of intravenous xylazine hydrochloride on blood glucose, plasma insulin and rectal temperature in neonatal foals. *Equine Veterinary Journal* 22(1), 43-47 1990
- Rogers RA, Tasat DR, Warner AE and Brain JD.** Quantitative recovery of pulmonary intravenous macrophages from sheep lungs. *Journal of Leukocyte Biology*. 56, 692-701, 1994
- Rooijen NV and Nieuwmegan RV,** Elimination of phagocytic cells in the spleen after intravascular injection of liposome-encapsulated dichloromethylene-diphosphonate: an enzyme histochemical study. *Cell Tissue Research* 283, 355-360, 1984
- Rorabeck CH.** Tourniquet-induced nerve ischaemia: an experimental investigation. *Journal of Trauma* 20(4), 280-286, 1980.
- Rosenow EC.** Drug-induced pulmonary disease. *Disease-a-Month* 40(5), 258-310 1994
- Roughton RD** Xylazine as an Immobilising agent for Captive White-Tailed Deer. *Journal of the American Veterinary Medical Association* 167(1) 574-576, 1975
- Roughan JV, Flecknell PA.** Effects of surgery and analgesic administration on spontaneous behaviour in singly housed rats. *Research in Veterinary Science* 69(3), 283-288, 2000
- Roughan JV, Flecknell PA.** Training in behaviour-based post-operative pain scoring in rats - An evaluation based on improved recognition of analgesic requirements. *Applied Animal Behav-*

- iour Science* 96(3-4) 327-342 2006
- Roughan JV, Wright-Williams SL & Flecknell PA.** Automated analysis of postoperative behaviour: assessment of HomeCageScan as a novel method to rapidly identify pain and analgesic effects in mice. *Laboratory Animals* 43(1), 17-26 2009
- Rudolph AM & Heyman AM.** The circulation of the fetus in utero: Methods of studying distribution of blood flow, cardiac output and organ blood flow. *Circulation Research* 21, 163-184 1967
- Rydevik B and Nordborg C.** Changes in nerve function and nerve fibre structure induced by acute, graded compression. *Journal of Neurology, Neurosurgery and Psychiatry* 43(12) 1070-82, 1980
- Schmitt H, Le Douarec JC & Petillot N.** Antagonism of the antinociceptive action of xylazine, an α -sympathomimetic agent, by adrenoceptor and cholinceptor blocking agents. *Neuropharmacology* 13(5), 295-303 1974
- Schwartz DD and Clark TP.** Affinity of detomidine, medetomidine and xylazine for alpha-2 adrenergic receptor subtypes. *Journal of Veterinary Pharmacology and Therapeutics* 21, 107-111, 1998a
- Schwartz DD & Clark TP.** Selectivity of atipamezole, yohimbine and toazoline for alpha-2 adrenergic receptor subtypes: implications for clinical reversal of alpha-2 adrenergic receptor subtypes. *Journal of Veterinary Pharmacology and Therapeutics* 21(5), 342-347 1998b
- Seksel, K.** Behavioural aspects of pain. Pain and practical pain therapy, *Proceedings from the AVERT and ANZCCART Conference*. Melbourne. 2001
- Selye H.** A Syndrome produced by Diverse Nocuous Agents. *Nature* 138, 32 1936
- Shirasaka T, Qui DL, Kannan H & Takasaki M.** The Effects of Centrally Administered Dexmedetomidine on Cardiovascular and Sympathetic Function in Conscious Rats. *Anesthesia and Analgesia* 105(6), 1722-1728 2007
- Silflow RM and Foreyt WJ** Susceptibility of phagocytes from elk, deer, bighorn sheep, and domestic sheep to *Pasteurella heamolytica* cytotoxins. *Journal of wildlife diseases* 30(4), 529-535, 1994
- Simon MA, Gielen MJ & Alberink N.** Intravenous regional anesthesia (IVRA) with 0.5% articaine, 0.5% lidocaine, or 0.5% prilocaine. A double-blind randomized clinical study. *Regional anesthesia* 22, 29-34 1997
- Simpson AM, Suttie GAM, Sharman GAM & Corrigan W.** Influence of some sedative drugs on the appetite of red deer. *Veterinary Record* 112, 385 1983
- Smith RH, Kano T, Cowan GSM & Barber RE.** Electroanesthesia (EA) Studies: Current Applications to Human Volunteers to Produce General Anesthesia and Local Anesthesia. *Anesthesia and Analgesia* 56, 678-685 1977
- Smith MC & Sherman DM.** Goat Medicine: Second edition (Wiley-Blackwell, Ames, Iowa, 2009).
- Sontakke SD, Reddy AP, Umapathy G & Shivaji S.** Anesthesia induced by administration of xylazine hydrochloride alone or in combination with ketamine hydrochloride and reversal by

- administration of yohimbine hydrochloride in captive Axis deer (*Axis axis*). *American Journal of Veterinary Research* 68(1), 20-24 2007
- Stafford KJ & Mellor DJ.** Dehorning and disbudding distress and its alleviation in calves. *The Veterinary Journal* 169, 337-349 2005
- Stafford KJ, Mellor DJ, Todd SE, Ward RN & McMeekan CM.** The effects of different combinations of lignocaine, ketoprofen, xylazine and tolazoline on the acute cortisol response to dehorning in calves. *New Zealand Veterinary Journal* 51(5), 219-226 2003
- Stafford KJ, Mellor DJ, Ward RN and Cann B.** Behavioural responses of calves to amputation dehorning with or without local anaesthesia. *Proceedings of the New Zealand Society of Animal Production* 60, 234-236, 2000
- Staub NC, Longworth KE, Serikov V, Jerome EH and Elsasser T.** Detergent inhibits 70-90% of responses to intravenous endotoxin in awake sheep. *Journal of Applied Physiology* 90, 1788-1797, 2001
- Stephens KE, Ishizaka A, Larrick JW & Raffin TA.** Tumor necrosis factor causes increased pulmonary permeability and edema comparison to septic acute lung injury. *American Journal of Respiratory Disease* 137(6), 1364-1370 1988
- Sterken J, Troubleyn J, Gasthuys F, Maes V, Diltor M & Verborgh C.** Intentional overdose of Large Animal Immobilon. *European Journal of Emergency Medicine* 11(5), 298-301 2004
- Stevens BJ & Franck LS.** Assessment and management of pain in neonates. *Paediatric Drugs* 3, 305-315, 2001
- Stewart MC & English AW.** The reversal of xylazine/ketamine immobilisation of fallow deer with yohimbine. *Australian Veterinary Journal* 67(9), 315-317 1990
- Storms TN, Schumacher J, Osborn DA, Miller KV & Ramsay EC.** Effects of ketamine on carfentanyl and xylazine immobilization of white-tailed deer (*Odocoileus virginianus*). *Journal of Zoo and Wildlife Medicine* 37(3), 347-353 2006
- Suttie JM, Chunyi L and Sadighi M.** Recent advances in antler physiology research. *Proceedings of a Deer Course for Veterinarians No.9, Deer Branch NZVA.* 111-118, 1992
- Suttie JM and Fennessy PF.** Regrowth of amputated velvet antler with and without innervation. *Journal of Experimental Zoology* 234, 359-66, 1985
- Suttie JM, Fennessy PF, Mackintosh CG, Corson ID, Christie R & Heap SW.** Sequential Cranial Angiography of Young Red Deer Stags. *Biology of Deer Production Bulletin* 22, 263-268 1985
- Suttie JM, Li C, Sheard PW, Corson ID and Waldrup KA.** Effects of unilateral cranial sympathectomy either alone or with sensory nerve sectioning on pedicle growth in red deer (*Cervus elaphus*) *Journal of Experimental Zoology* 271(2) 131-138, 1995
- Suzuki M, Nakamura Y, Onuma M, Tanaka J, Takahashi H, Kaji K & Ohtaishi N.** Acid-base Status and Blood Gas Arterial Values in Free-Ranging Sika Deer Hinds Immobilised with Medetomidine and Ketamine. *Journal of Wildlife Diseases* 37(2), 366-369 2001
- Sylvester SP, Stafford KJ, Mellor DJ, Bruce RA and Ward RN.** Behavioural responses of calves to amputation dehorning with or without local anaesthesia. *Australian Veterinary Journal* 82

- (11), 697-700, 2004
- Symonds HW & Mallinson CB.** The effect of xylazine and xylazine followed by insulin on blood glucose and insulin in the dairy cow. *Veterinary Record* 102(2), 27-29 1978
- Taylor JS & Vierck CJ.** Effects of Ketamine on electroencephalographic and autonomic arousal and segmental reflex responses in the cat. *Veterinary Anaesthesia and Analgesia* 30, 237-249 2003
- Theurer ME, White BJ, Coetzee JF, Edwards NL, Mosher RA and Cull CA.** Assessment of behavioural changes associated with oral meloxicam administration at time of dehorning calves using a remote triangulation device and accelerometers. *BMC Veterinary Research* 8(48)
- Thomas JW, Robinson RM & Marburger RG.** Use of diazepam in the capture and handling of cervids. *Journal of Wildlife Management* 31(4), 686- 1967
- Thompson JR, Hsu WH & Kersing KW.** Antagonistic effect of idazoxan on xylazine-induced central nervous system depression and bradycardia in calves. *American Journal of Veterinary Research* 50(5), 734-736 1989
- Thornton PD, Waterman-Perason AE.** Behavioural responses to castration in lambs. *Animal Welfare* 11(2) 203-212 2002
- Thurmon JC, Steffey EP, Zinkl JG, Wolimer M & Howland DJ.** Xylazine causes transient dose-related hyperglycemia and increased urine volumes in mares. *American Journal of Veterinary Research* 45(2), 224-227 1984
- Tom EM, Duncan IJH, Widowski TM, Bateman KG and Leslie KE** Effects of tail docking using a rubber ring with or without anaesthetic on behaviour and production of lactating dairy cows. *Journal of Dairy Science* 85(9) 2257-2265 2002
- Tompsett DH & Wyke B.** A method for preparing corrosion casts of the cerebral arterial system of laboratory animals in the comparative study of the cerebral vascular system. *Acta. Anat (Basel)* 72(3), 460-475 1969
- Tranquilli WJ, Thurmon JC, Grimm KA, Lumb WV** eds. In Lumb and Jones' veterinary anaesthesia and analgesia 4th edition, Blackwell Publishing 2007
- Tranquilli WJ, Thurmon JC, Neff-Davis CA, Davis LE, Benson GJ, Hoffman W & Lock TF.** Hyperglycemia and hypoinsulinemia during xylazine-ketamine anesthesia in Thoroughbred horses. *American Journal of Veterinary Research* 45(1), 11-14 1984
- Tsigos C & Chrousos GP.** Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research* 53, 865-871 2002
- Tyde'n E, Tjälve H and Larsson P.** Metabolic Activation of 2,6-Xylidine in the Nasal Olfactory Mucosa and the Mucosa of the Upper Alimentary and Respiratory Tracts in Rats, *Toxicological Science* 81, 263-272, 2004
- Uggla A and Lindqvist A.** Acute pulmonary oedema as an adverse reaction to the use of xylazine in sheep. *The Veterinary Record*, 113 p42 1983
- Vacek Z.** Innervace lyci rostoucia parohu u cervidu. *Ceskoslovenska Morfologie* 3: 249-246. 1955
- Van der Eems K & Brown RD.** Effect of caffeine sodium benzoate, ketamine hydrochloride, and

- yohimbine hydrochloride on xylazine hydrochloride-induced anorexia in white-tailed deer. *Journal of Wildlife Diseases* 22(3), 403-406 1986
- van Oosterhout MFM, Prinzen FW, Sakurada S, Glenny RW & Hales JRS.** Fluorescent microspheres are superior to radioactive microspheres in chronic blood flow measurements. *American Journal of Physiology Heart and Circulatory Physiology* 275 (1), 110-115 1998
- van Oosterhout MFM, Willigers HM, Reneman RS & Prinzen FW.** Fluorescent microspheres to measure organ perfusion: validation of a simplified sample processing technique. *American Journal of Physiology* 269, 725-733 1995
- Verbeke AJ & Viaene J.** Ethical challenges for livestock production: Consumer concerns about meat safety and animal welfare. *Journal of Agricultural and Environmental Ethics* 12(2) 2000
- Virtanen R & Nyman L.** Evaluation of the alpha 1- and alpha 2-adrenoceptor effects of detomidine, a novel veterinary sedative analgesic. *European Journal of Pharmacology* 108(2), 163-169 1985
- Virtanen R, Savola J-M, Saano V & Nyman L.** Characterization of the selectivity, specificity and potency of medetomidine as an α 2-adrenoceptor agonist. *European Journal of Pharmacology* 150, 9-14 1988
- Vree TB & Gielen MJ.** Clinical pharmacology and the use of articaine for local and regional anaesthesia. *Best Practice and Research Clinical Anaesthesiology* 19 (2), 293-308 2005
- Vree TB, Simon MA, Gielen MJ & Booij LHDJ.** Regional metabolism of articaine in 10 patients undergoing intravenous regional anaesthesia during day case surgery. *British Journal of Clinical Pharmacology* 44(1), 29-34 1997
- Wagner AE, Muir WWr & Hinchcliff KW.** Cardiovascular effects of xylazine and detomidine in horses. *American Journal of Veterinary Research* 52(5), 651-657 1991
- Waldo CM, Wislocki GB, Fawcett DW.** Observations on the blood supply of growing antlers. *American Journal of Anatomy* 84: 27-61. 1949
- Walker IH.** Update of 1988 Survey data. *Proceedings of a Deer Course for Veterinarians, No.6*, Deer Branch of the New Zealand Veterinary Association: 145-150. 1989
- Walker IH and Middelberg A.** Post-velvet deaths in stags. *Proceedings of a Deer Course for Veterinarians* Deer branch of the NZVA, 5, 128-134, 1988
- Wall PD.** On the relation of injury to pain - the John J Bonica lecture. *Pain* 6, 253-264, 1979
- Wallingford BD, Lancia RA & Soutiere EC.** Antagonism of xylazine in white-tailed deer with intramuscular injection of yohimbine. *Journal of Wildlife Diseases* 32(2), 399-402 1996
- Walsh VP and Wilson PR.** Sedation and chemical restraint of deer. *New Zealand Veterinary Journal*, 50(6) 228-236, 2002
- Warren JB, Dollery CT, Sciberras D & Goldberg MR.** Assessment of MK-467, a peripheral alpha-2 adrenergic receptor antagonist, with intravenous clonidine. *Clinical Pharmacology and Therapeutics* 50(1), 71-77 1991
- Warren RJ, Kirkpatrick RL, Gibson DF & Scanlon PF.** Xylazine hydrochloride-induced anorexia in white-tailed deer. *Journal of Wildlife Diseases* 20, 66-68 1984

- Waterman AE, Nolan A and Livingston A.** Influence of idazoxan on the respiratory blood gas change induced by α_2 -adrenoceptor agonist drugs in conscious sheep. *Veterinary Record* 121, 105-107, 1987
- Webster JR, Bremner KJ, Pearse AJ, Morrow CJ and Matthews LR.** Evaluation of a compression method of producing analgesia for velvet antler removal in red deer. *Proceedings of a Deer Course for Veterinarians No.19* Deer branch of the NZVA, 137-143, 2002
- Webster JR and Matthews LR.** Behaviour of red deer following antler removal with two methods of analgesia. *Livestock Science* 100, 150-158, 2006
- Webster JR, Matthews LR, Cook NJ & Schaefer AL.** Welfare assessment of a non-chemical method of providing analgesia for humane velvet removal. *2nd International Symposium on Antler Science and Product Technology* 65-73 2005
- Webster, J, Matthews L, Schaefer A and Cook N.** Compression for Velvet Antler Analgesia: Humaneness and Practical Applications. *Proceedings of a Deer Course for Veterinarians*, Queenstown, pp. 69-78. 2003
- Welch PD.** The use of Fast Fourier Transformation for the estimation of power spectra: a method based on time averaging over short, modified periodograms. *IEEE Trans Audio Electroacoustics* 15, 70-73, 1967
- Welsh EM, Nolan AM.** Effects of nonsteroidal antiinflammatory drugs on the hyperalgesia to noxious mechanical stimulation-induced by the application of a tourniquet to a forelimb of sheep. *Research in Veterinary Science* 57(3) 285-291 1994
- West NO & Nordan HC.** Hormonal regulation of reproduction and the antler cycle in the male columbian black-tailed deer (*Odocoileus hemionus columbianus*). Part I. Seasonal changes in the histology of the reproductive organs, serum testosterone, sperm production and the antler cycle. *Canadian Journal of Zoology* 54(10), 1617-1636 1976
- Wild RJ.** Regulatory issues and deer slaughter. *Proceedings of the deer branch of the New Zealand Veterinary Association*. 25, 79-82, 2008.
- Wilson PR, Beimans J, Stafford KJ, Veltmain CJ & Spoorenberg J.** Xylazine and a xylazine/fentanyl citrate/azaperone combination in farmed deer I: Dose rate comparison. *New Zealand Veterinary Journal* 44(3), 81-87 1996a
- Wilson PR, Biemans J, Stafford KJ, Veltman CJ and Spoorenberg J.** Xylazine and xylazine/fentanyl citrate/azaperone combination in farmed deer II: Velvet antler removal and reversal combinations *New Zealand Veterinary Journal* 44, 88-94, 1996b
- Wilson PR & Pauli JV.** Blood constituents of farmed red deer (*Cervus elaphus*) I. Haematological values. *New Zealand Veterinary Journal* 30(11), 174-176 1982
- Wilson P, Stafford K, Thomas D, Mellor D.** Evaluation of techniques for lignocaine analgesia of the velvet antler of adult stags. *New Zealand Veterinary Journal* 48, 182-187, 2000
- Wilson, P., Thomas, D., Stafford, K., Mellor, D.** Routes and doses of lignocaine hydrochloride for analgesia of the velvet antler of stags. *New Zealand Veterinary Journal* 47, 167-174. 1999
- Wislocki G & Singer M.** The occurrence and function of nerves in the growing antlers of deer. *Journal of Comparative Neurology* 85, 1-16 1946

- Woodbury MR, Caulkett NA, Baumann D, Read MR.** Comparison of analgesic techniques for antler removal in wapiti. *Canadian Veterinary Journal* 42, 929-935 2001
- Woodbury MR, Caulkett NA, Johnson CB, and Wilson PR.** Comparison of analgesic techniques for antler removal in minimally anaesthetised red deer (*Cervus elaphus*): Cardiovascular and somatic responses. *Veterinary Anaesthesia and Analgesia*, 32: 72-82, 2005
- Woodbury MR, Caulkett NA, & Wilson PR.** Comparison of lidocaine and compression for velvet antler analgesia in wapiti. *Canadian Veterinary Journal* 43, 869-875, 2002
- Woodbury MR & Haigh JC** Innervation and anaesthesia of the antler pedicle in wapiti and fallow deer. *Canadian Veterinary Journal* 37, 486-489, 1996
- Yamashita K, Tsubakishita S, Futaok S, Hamaguchi H, Seno T, Katoh S, Izumisawa Y, Kotani T & Muir WW.** Cardiovascular effects of medetomidine, detomidine and xylazine in horses. *Journal of the Veterinary Medical Society* 62(10), 1025-1032 2000

Appendix 5

5.2 Hypoxaemia in weaner red deer following sedation with xylazine.

Introduction

The results presented in the previous section showed that pulmonary oedema and cellular infiltration are common postmortem and histological findings in stags that have died following sedation with xylazine. Hypoxaemia has been described in several species of deer and in sheep and cattle after administration of alpha 2 agonists and is reviewed in Chapter 1.5

This chapter investigated the effect of xylazine sedation on the haemoglobin oxygen saturation, partial pressure of arterial oxygen and haematological parameters in rising one year old red deer during teaching practical classes at the Massey University Deer Farm. The aims of these observations were to determine the extent and variation in effective oxygenation of the blood following sedation with xylazine alone in red deer and to investigate circulating cellular changes that may indicate an inflammatory or immune mediated response to xylazine.

It is not known whether xylazine affects all deer in the same way but to varying levels such that some are susceptible to dying due to predisposing factors such as genetics or underlying lung pathology or whether the majority of deer do not have an adverse response to xylazine but a few have a fatal reaction for unknown reasons. This study also aimed to determine whether normal deer that do not show any obvious signs of respiratory distress or death also suffer from pulmonary compromise.

The results show that the majority of deer experience severe depression in blood oxygen levels, particularly the concentration of oxygen carried on the haemoglobin and that there is a large variation between animals in this response. These results are suggestive that deaths are due to severe depression in oxygen exchange in highly susceptible animals that may also have an underlying pulmonary compromise. This is in contrast to the generally held belief that stag death was due to an anaphylactic reaction in susceptible animals.

Material and methods

One hundred and seventy eight rising one year old red deer were sedated by 4th year BVSc students at Massey University from 2006 to 2011 inclusive under the supervision of the author of this thesis. Students were given record sheets to fill in and instructions on monitoring the animals during sedation.

The deer were a mixture of entire males (N=117) and females (N=61) ranging in weight from 53.5 to 117kg (mean=77.1, SD=9.9). All deer, except those used during 2007 were born and reared at the Massey University Deer Teaching Unit. Deer used during 2007 were bought in for another project.

For each deer, the animal was weighed and a pre-anaesthetic clinical examination was conducted. When possible, attitude and demeanour, body condition score, coat condition, lung auscultation, respiratory rate, cardiac auscultation, heart rate and rhythm, mucous membrane colour and capillary refill time and rectal temperature were measured. A small number (N=16) had a pulse oximeter reading of SpO₂ taken prior to sedation.

After determining that the animal was without obvious disease or pathology and thus a suitable candidate for sedation, either xylazine (Phoenix xylazine injection, Phoenix Pharm Distributors Ltd., Auckland, NZ; 2, 5 or 10mg/mL) or detomidine (Dormosedan, Pfizer NZ Ltd. Auckland, NZ, 10mg/mL) (15 deer) was administered either intravenously (N=138) or intramuscularly (N=29). Three students attempted IV injection but missed the vein and 8 did not state the route used. The most common dose rate for xylazine was either 0.5mg/kg intravenously into the jugular vein or 1.0mg/kg intramuscularly into the anterior part of the neck and for detomidine was 0.1mg/kg intravenous. After sedation, each animal was moved to a pen and allowed to lie down on a rubber mat. A pulse oximeter was placed on the ear or tongue as soon as it would be tolerated. Time to recumbency was recorded and physiological measurements were made as soon as practical. These included heart rate, respiratory rate, arterial haemoglobin oxygen saturation (pulse oximeter), mucous membrane colour and capillary refill time. Recordings were made every two to three minutes. After a minimum of ten minutes sedation, either yohimbine (Reversal injection, Phoenix Pharm Distributors Ltd, Auckland, NZ) or tolazoline (Tolazine injection; Lloyd Laboratories NZ Ltd) was administered intravenously into the jugular vein or a catheter placed in the marginal ear vein at a dose of 0.2mg/kg or 2mg/kg for yohimbine or tolazoline, respectively. Time to recovery after reversal was recorded and students were instructed to continue to take measurements until the stag was standing and would no longer tolerate recordings.

Haematology

Eight deer had venous blood samples taken from the jugular vein prior to and 5 minutes after intravenous injection of xylazine to assess if the herd had normal parameters. These were taken into EDTA tubes, kept in a refrigerator overnight and submitted to the New Zealand Veterinary Pathology Laboratory at Massey University the following day for analysis. Red blood cell count, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, white cell count, segmented neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, and fibrinogen were measured.

Blood gas analysis

Forty-six animals had arterial blood samples taken 5 minutes after injection of xylazine for blood gas analysis. Samples were analysed immediately. The first samples in 2008 were analysed using istat and cg4+ cartridges (Istat Abbott Laboratories, 400 College Road East, Princetown, NJ 08540, USA.) In 2009 an idexx vetstat was used with Respiratory/blood gas cassettes. Idexx (Idexx laboratories Inc. Westbrook, Maine 04092, United States).

pH, PaCO₂, HCO₃⁻, tCO₂, PaO₂, SO₂ and Base Excess were determined and were compared to

expected normal ranges (Bouda et al 2000; Paterson et al, 2009).

TNF alpha assays

16 Animals had venous blood samples taken using a red top (plain) blood tube prior to sedation and 5 minutes after injection of xylazine for TNF alpha assay. Samples were centrifuged and the serum removed and stored at -80°C until a test had been validated by a PhD student at the University of Otago Deer Research Laboratory. When the test was available samples were sent on ice to Otago University for testing using an ELISA test for TNF alpha with bovine antibodies and cross reaction of deer serum TNF alpha with bovine TNF alpha antibodies from AbD seroTec Ltd (UK and worldwide, sales@abdirect.com, www.abdserotec.com.)

Statistical analysis

Results were analysed using Microsoft excel and Prism 5 software. Two sample student t-tests were used to compare deer that were treated with xylazine versus detomidine and also to compare male versus female deer, intramuscular versus intravenous route of injection and pre versus post sedation values where they were recorded. When repeated measurements were made over time, one way ANOVA with post ANOVA Tukey's comparisons between each time interval were done without repeated measures as not all deer had repeated values at each time point. For haematological data, paired t-tests were used to compare pre-sedation and during sedation values because all eight deer had pre and post sedation values.

Results

Data collection was not complete for most animals. Where applicable, the number of usable data points for each treatment or result is indicated.

Male vs Female

There were 117 male deer and 61 female deer used in the study. Male deer were significantly heavier than female deer and were given a significantly higher volume of sedative and reversal drug. Apart from that there were no significant differences between male and female deer in any of the parameters measured. Therefore data for male and female deer were combined.

Time to recumbency

Seventy five deer had the time from injection to recumbency recorded. Deer given xylazine intravenously (N=57) took 3.7 ± 4.7 (SD) minutes to become recumbent. Deer given xylazine intra-

muscularly (N=15) took 7.9 ± 3.4 minutes to become recumbent. This difference was significant ($p=0.002$). 34/57 deer were recumbent within 2 minutes of intravenous injection. 7/57 deer given xylazine intravenously took 10 or more minutes to become recumbent.

Time to standing after reversal

Fifty six deer had the time from injection of reversal agent to standing recorded. The mean and standard deviation of time to standing after reversal was 7.1 ± 5.9 minutes. The range was 1 to 26 minutes. Deer that had been injected with xylazine intramuscularly (N=12) took significantly longer ($p<0.0001$) to recover than deer injected intravenously (N=44). Mean and SD were 13.8 ± 6 and 5.3 ± 4.4 minutes for intramuscular and intravenous injection, respectively.

Heart rate

Heart rate prior to injection of xylazine was recorded in 153 animals. The mean and standard deviation of heart rate prior to sedation was 81 ± 16 beats per minute.

Xylazine and detomidine caused a decrease in heart rate. The mean heart rate during sedation with xylazine was 62 ± 9.3 beats per minute (range 43 to 104) for 132 deer that had their heart rates recording during sedation. The mean heart rate during sedation with detomidine was 48 ± 12 bpm (range 35 to 72) for 8 deer that had their heart rates recorded during sedation with detomidine. This difference was statistically significant ($p<0.001$).

There was no difference in heart rate during sedation for deer that had been injected with xylazine via the intravenous route compared to the intramuscular route. Figure 5.2.1 shows the mean and 95% confidence interval of the mean with individual data points for each deer recorded before sedation and during each 2 to 3 minutes after injection of xylazine or detomidine. The number of deer that had heart rate recordings at each time interval is shown.

Heart rate did not change significantly over time during sedation.

Respiratory rate

Respiratory rate prior to sedation was recorded in 129 animals. The mean respiratory rate prior to sedation was 45 ± 25 breaths per minute (range 12 to 120). Respiratory rates at the high end of this range could be considered as panting.

After injection of xylazine or detomidine, respiratory rate decreased significantly. The mean respiratory rate during sedation was 18 ± 13 (range 4 to 74) breaths per minute for the 129 deer that had respiratory rates recorded during sedation. The mean and 95% confidence interval of the mean and individual animal values are shown for pre sedation and each 2 to 3 minutes during sedation in Figure 5.2.2. The numbers above each time interval are the number of deer for which recordings were made at that time.

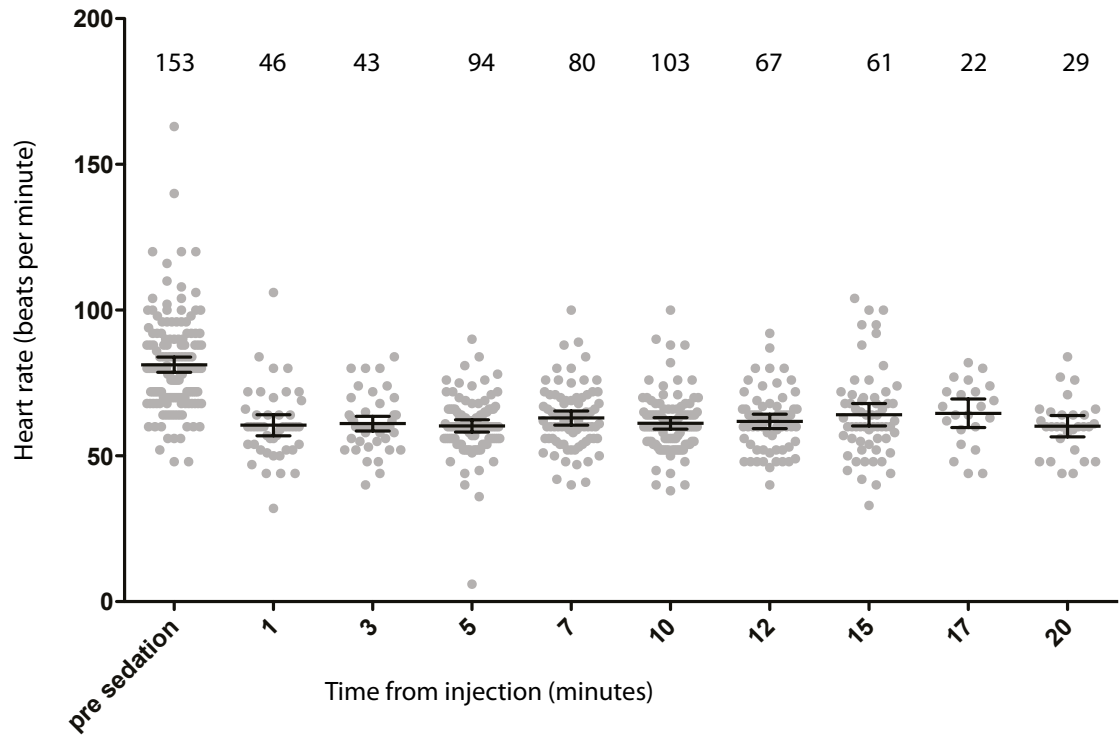


Figure 5.2.1 Heart rate of rising one year old red deer recorded before sedation and during sedation with xylazine or detomidine. Numbers above each time interval indicate the number of animals from which recordings were made at each time. Lines and error bars are the mean and 95% confidence interval of the mean.

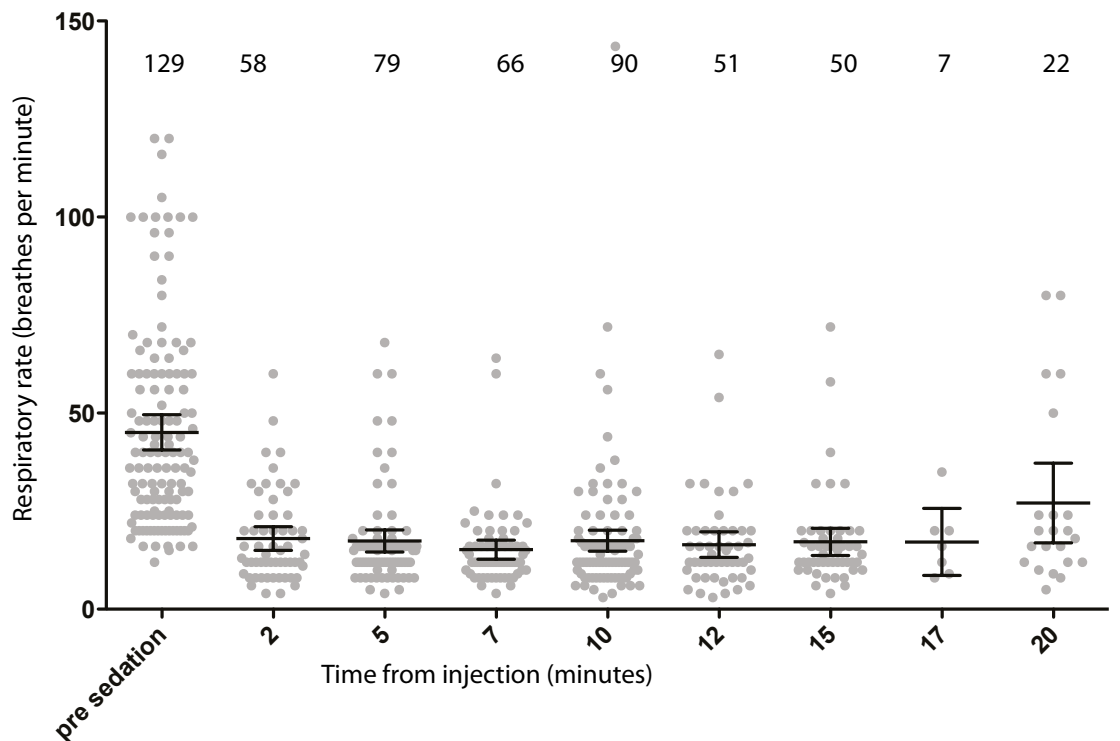


Figure 5.2.2 Respiratory rate of rising one year old red deer recorded before sedation and during sedation with xylazine or detomidine. Numbers above each time interval indicate the number of animals from which recordings were made at each time. Lines and error bars are the mean and 95% confidence interval of the mean

There was no difference in respiratory rate between deer that had been injected with xylazine or detomidine or for deer that had been injected by the intravenous versus the intramuscular route.

Arterial haemoglobin oxygen saturation

The mean and 95% confidence interval of the mean of SpO₂ and individual values recorded for each animal prior to sedation, at various time intervals after sedation and after reversal are shown in Figure 5.2.3. The numbers above the time (x-axis) are the number of animals for which recordings were made at that time. Only one deer that was sedated with detomidine had a pulse oximeter recording made and was not included in these data.

Pre sedation

Prior to sedation SpO₂ was measured in 16 animals by placing the probe on the cranial or caudal branch of the auricular artery at the respective proximal corner of the auricular cartilage near the base. The mean SpO₂ was 93% ± 3% with a range of 88 to 99%.

During sedation

During sedation, SpO₂ was recorded in 124 animals. The mean SpO₂ was 75% ± 12.6% with a range of 24 to 97% across all recordings. This was significantly lower than recordings made prior to sedation (unpaired student t test, $p < 0.0001$).

The minimum SpO₂ recorded for individual deer during sedation is shown in Figure 5.2.4. The mean ± standard deviation of the minimum SpO₂ was 68 ± 16% (range 24-92). 119/124 deer had SpO₂ readings of less than 90% at some time during sedation and all were below 92% at some time.

After reversal

Thirty-nine animals had their SpO₂ recorded immediately after giving reversal agent. The mean and standard deviation was 89 ± 6.5% with a range of 72 to 99. This was significantly lower than the pre sedation recordings ($p=0.032$) and was significantly higher than recordings made during sedation ($p<0.0001$).

Effect of time during sedation

One-way ANOVA of SpO₂ during sedation found a significant effect of time. Post-ANOVA Tukey's test showed that SpO₂ at 2 and 5 minutes was lower than at 12 and 15 minutes. There was a trend for SpO₂ to increase over time with the lowest levels 2 minutes after injection of xylazine.

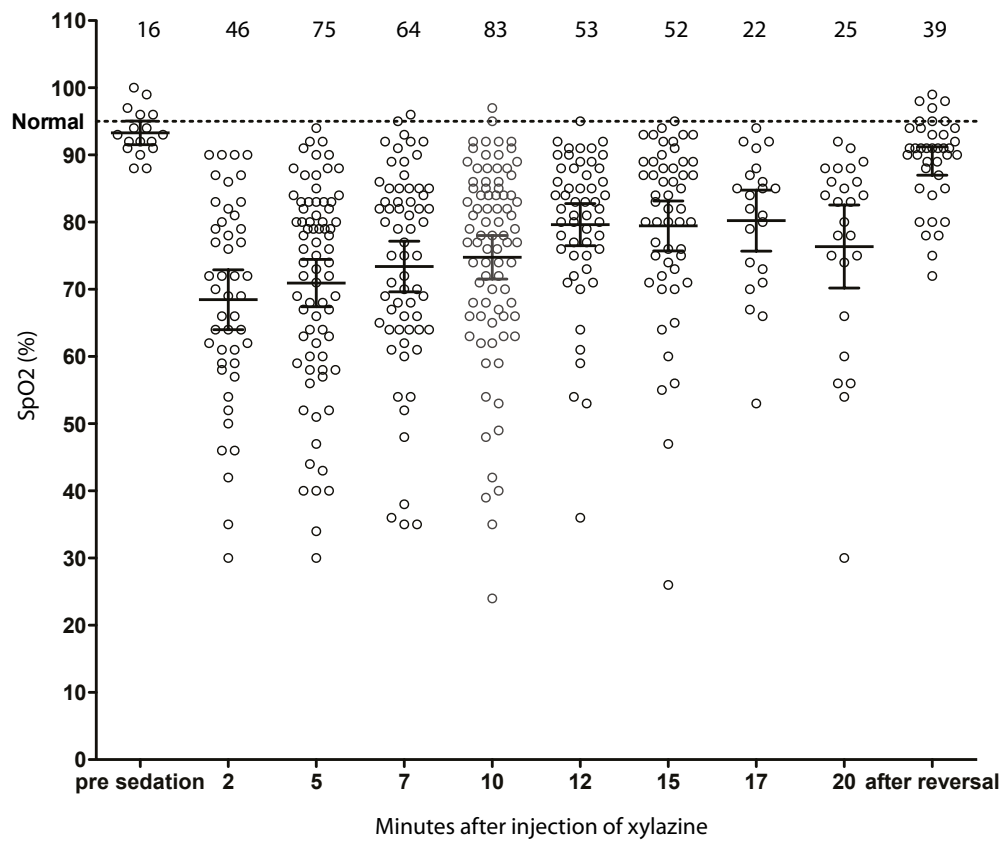


Figure 5.2.3 (above) SpO_2 measured using a pulse oximeter in rising one year old deer prior to and at various time intervals during sedation with xylazine and after reversal of sedation. Line is at the mean and error bars are 95% CI of the mean.

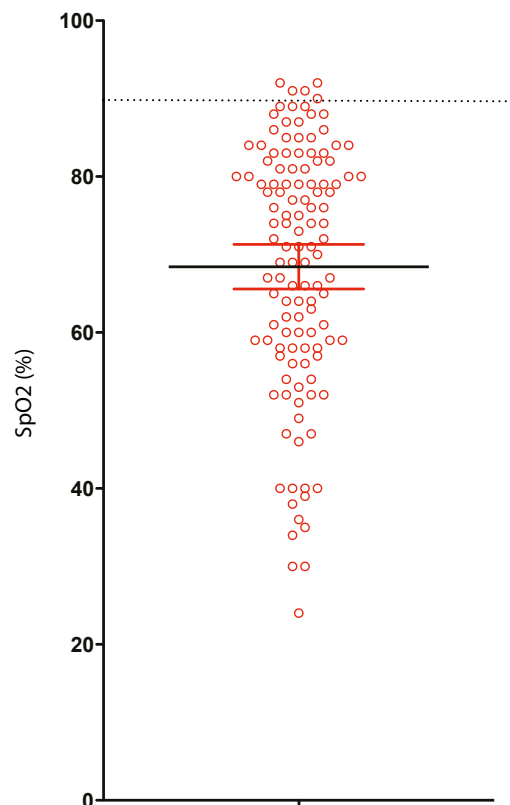


Figure 5.2.4 (left) Minimum SpO_2 measured using a pulse oximeter in rising one year old deer during sedation with xylazine. Line is at the mean and error bars are 95% CI of the mean. Level that an animal is considered to be hypoxaemic and oxygen should be provided is shown at a dotted line.

Intramuscular vs Intravenous

Of the animals that were injected via the intramuscular route, 18 had SpO₂ recorded at least once during sedation and of the animals injected via the intravenous route, 98 had SpO₂ recorded during sedation. Deer injected intramuscularly had significantly higher mean SpO₂ recorded than deer injected intravenously ($p=0.045$). The mean and standard deviation of the mean SpO₂ during sedation for deer injected intramuscularly was $80 \pm 9.6\%$ (range 60-92) and for deer injected intravenously was $74 \pm 13\%$ (range 26.6 to 91.5).

The minimum SpO₂ recorded for each animal during sedation was significantly lower for deer injected via the intravenous route compared to the intramuscular route ($p<0.0001$). Mean and SD of the minimum SpO₂ following intramuscular injection was $78 \pm 11\%$ (range 56-92) and following intravenous injection was $67 \pm 17\%$ (range 24-91).

Arterial Blood Gas Analysis

Arterial blood gas readings are summarised in Table 5.2.1. All except for one deer was male and all were injected intravenously. Thirteen animals were injected with detomidine and 33 were injected with xylazine. PaO₂, PaCO₂, SaO₂, tCO₂, pH, HCO₃⁻ and base excess are shown for each deer sedated with either xylazine or detomidine in Figure 5.2.5. Expected normal ranges are plotted on each graph panel.

PaO₂

PaO₂ was low compared to the expected normal range in all but one animal that was sedated with xylazine and was less than 60mmHg in 35/46 deer indicating that oxygen supplementation should be administered. PaO₂ was significantly lower in deer sedated with xylazine compared to deer sedated with detomidine ($p<0.0001$).

PaCO₂

PaCO₂ was increased above expected normal levels in some deer that were sedated with xylazine but not in deer that were sedated with detomidine. This difference was statistically significant ($p=0.0008$).

pH

Mean pH was significantly higher in deer that had been sedated with detomidine than deer sedated with xylazine ($p=0.0002$).

Table 5.2.1 Arterial blood gas analysis results from rising one year old red deer 5 minutes after intravenous injection of xylazine or detomidine.

Parameter		N	Mean	StDev	min	max	Sig diff?
TCO ₂	overall	43	28.4	2.3	24	32.3	
	xylazine	31	28.6	2.3	24	32	No
	detomidine	12	27.7	2.4	24.4	32.3	
PaCO ₂	overall	43	43.5	3.9	34.0	53.0	
	xylazine	31	44.7	3.6	34.0	53.0	Yes p=0.0008
	detomidine	12	40.4	2.9	35.0	45.0	
PaO ₂	overall	46	52.5	12.4	32	83	
	xylazine	33	48.3	10.4	32	83	Yes p<0.0001
	detomidine	13	63.3	10.8	43	78	
SaO ₂ (%)	overall	45	84.9	8.8	60	97	
	xylazine	33	82.0	8.2	60	97	yes p<0.0001
	detomidine	12	92.9	3.8	83	96	
pH	overall	43	7.42	0.046	7.33	7.52	
	xylazine	31	7.41	0.039	7.33	7.48	Yes p=0.0003
	detomidine	12	7.46	0.042	7.4	7.52	
HCO ₃	overall	43	27.1	2.4	23	31.1	
	xylazine	31	27.2	2.3	23	31	No
	detomidine	12	26.7	2.4	23.3	31.1	
Base Excess	overall	44	3.3	2.8	-2	8.91	
	xylazine	32	2.9	2.7	-2	7.83	No
	detomidine	12	4.1	2.8	0.11	8.91	
tHb g/dL	overall	25	14.3	1.1	12.3	16.7	
	xylazine	13	14.8	0.8	13.6	16.5	p=0.038
	detomidine	12	13.8	1.2	12.3	16.7	
Anion Gap	overall	24	12.3	6.6	4.6	40.2	
	xylazine	12	11.1	3.2	4.6	15.8	No
	detomidine	12	13.5	8.8	6.1	40.2	
Sodium	overall	25	132.6	7.5	109	144	
	xylazine	13	134.6	5.6	119	144	No
	detomidine	12	130.4	8.8	109	141	
Potassium	overall	25	3.7	0.4	3.1	4.8	
	xylazine	13	3.7	0.3	3.2	4.2	No
	detomidine	12	3.6	0.5	3.1	4.8	
Chloride	overall	41	94.0	13.8	28	109	
	xylazine	29	94.1	13.8	28	109	No
	detomidine	12	93.8	14.5	50	104	

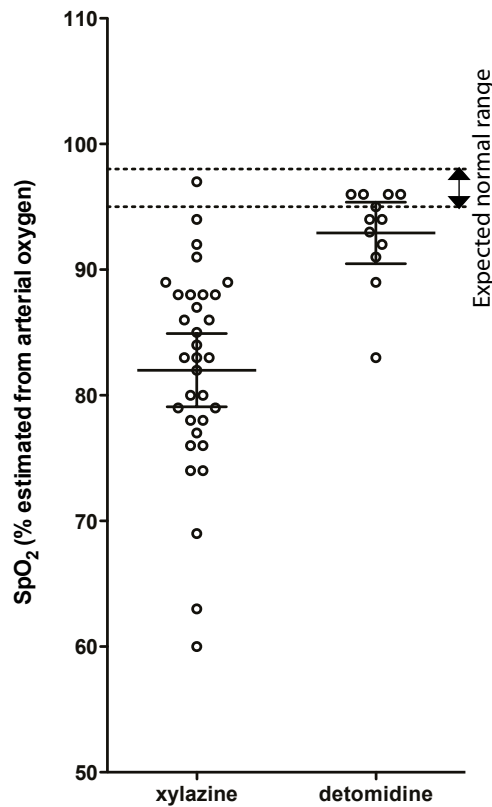
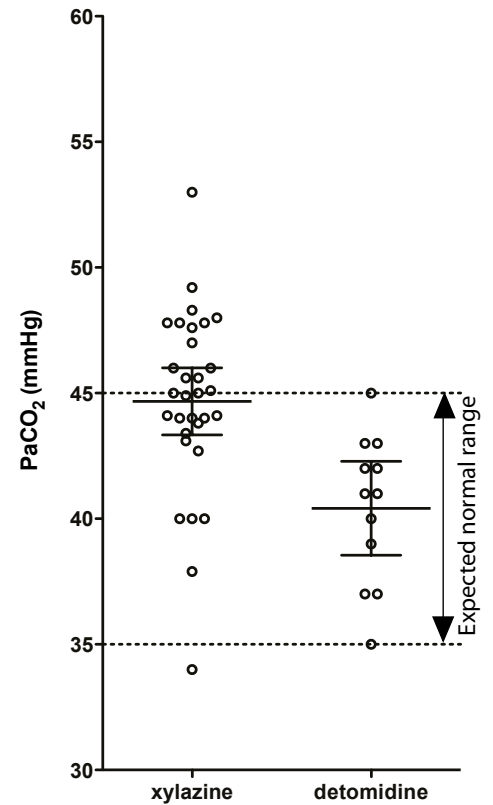
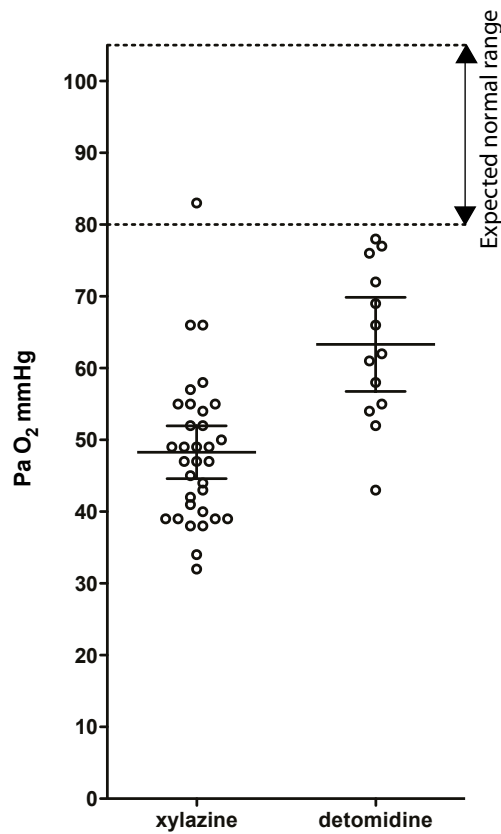
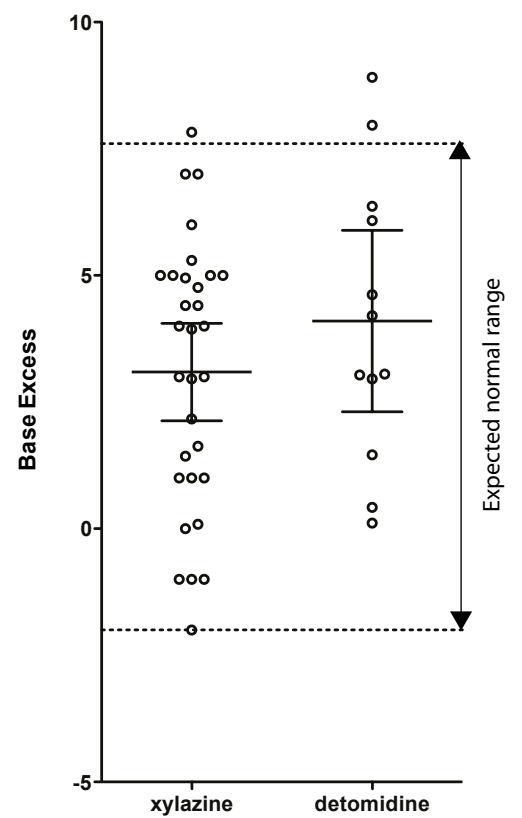
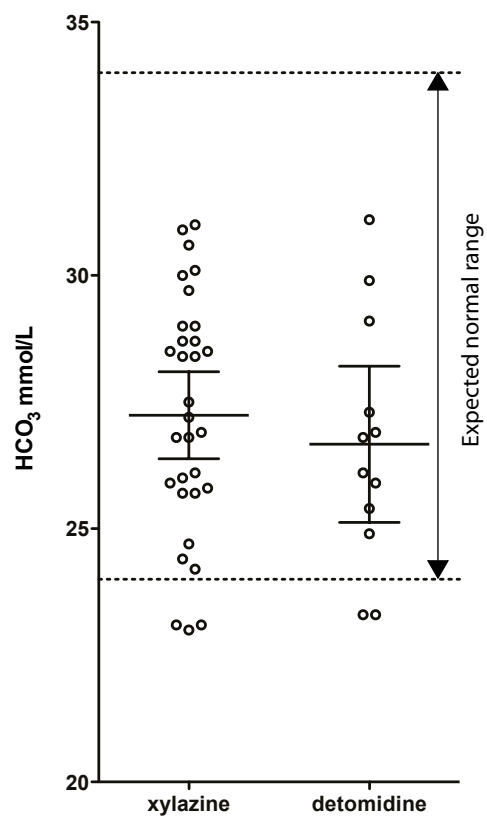
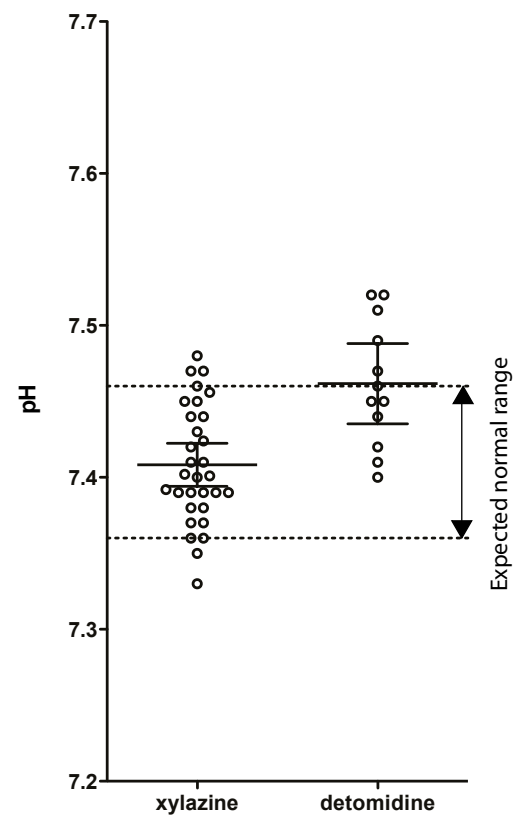
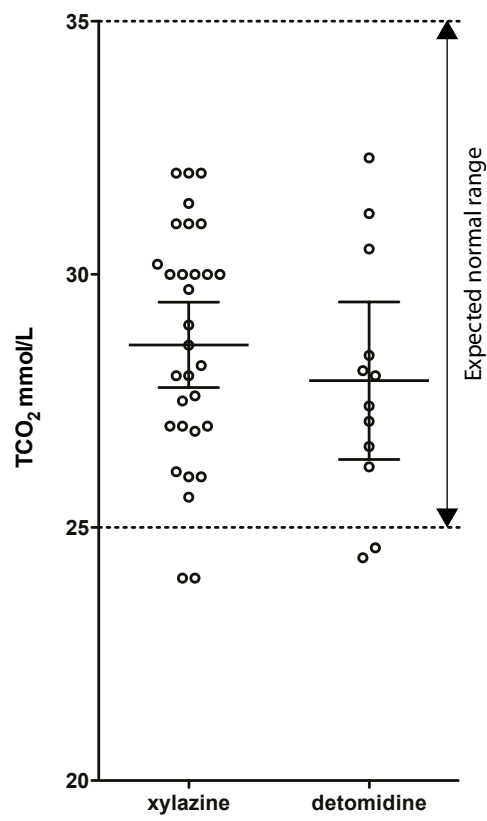


Figure 5.2.5 (this and the following page)
Arterial blood gas analysis from the auricular artery of yearling deer 5 minutes after sedation with either xylazine or detomidine.

PaO_2 , PaCO_2 , and SaO_2 are shown on this page and TCO_2 , pH, HCO_3^- and base excess are shown on the following page. Dotted lines indicate the expected range for normal, healthy, unsedated deer estimated from the literature (Bouda et al, 2000; Paterson et al (2009) and laboratory reference guides where available.



SaO₂

Haemoglobin oxygen saturation was lowered from expected normal levels in 32/33 deer sedated with xylazine and in all except for 8/12 deer sedated with detomidine. SaO₂ was significantly lower in deer sedated with xylazine than deer sedated with detomidine ($p < 0.0001$).

HCO₃⁻

Bicarbonate was within the expected normal range for most deer with 3 in the xylazine group and 2 in the detomidine group slightly lower than the expected minimum. There was no difference between deer sedated with xylazine or detomidine.

Base excess

Base excess was within the expected range for most deer and there was no difference between xylazine and detomidine in base excess.

Haematology

Venous blood sample CBC, WCC and differential results from samples taken prior to and 5 minutes after intravenous injection of xylazine are shown for each deer in Table 5.2.2. Figure 5.2.6 shows values for the eight individual deer for samples taken prior to and during sedation. Also shown are reference ranges estimated from Cross et al (1989) and Wilson and Pauli (1982).

All individual deer had lower values for haematocrit, red blood cell count, white blood cell count and lymphocytes during sedation compared to pre sedation. These parameters, along with segmented neutrophils and basophils had a significantly lower means (paired t-test) during sedation compared to pre sedation.

There were no significant differences in mean monocyte or eosinophil count or fibrinogen.

TNF alpha

TNF alpha results are shown in Table 5.2.3 for individual deer for samples taken pre sedation and during sedation with intravenous xylazine. There was no significant difference in the mean TNF alpha for pre sedation and during sedation samples. There was a wide range of values between animals and the data were not normally distributed and were skewed to the right. Within each animal there was a strong correlation between values pre and during sedation. Spearman R = 0.9382.

Haptoglobin

4 samples taken during sedation were analysed for haptoglobin by John Cross, Otago University. The results were 1.44, 2.57, 0.24 and 0.29 mg/mL for these 4 deer. The reference range is 0.02-0.18 (Cross et al, 1988).

Table 5.2.2 Complete blood count results from rising one year old deer prior to (pre) and during sedation with intravenous xylazine as part of veterinary student teaching practical classes.

Parameter	Mean		Standard deviation		paired t-test pre vs during
	pre	during	pre	during	
HCT	0.44	0.37	0.04	0.03	p=0.0001
RBC ($\times 10^{12}$ cell/L)	11.8	10.0	1.24	0.79	p=0.0003
Haemoglobin	172	146	14.4	9.8	p=0.0002
MCV	37.0	37.4	2.0	2.0	p=0.08
MCH	14.875	14.875	0.83	0.83	p=1
WCC	8.34	6.86	0.96	0.87	p=0.0016
seg neutrophils	4.30	3.84	0.73	0.73	p=0.042
lymphocytes	3.38	2.51	0.49	0.70	p=0.0005
monocytes	0.31	0.21	0.11	0.14	p=0.1
eosinophils	0.14	0.21	0.05	0.28	p=0.50
basophils	0.24	0.21	0.02	0.12	p=0.02
fibrinogen	4.75	4.38	0.83	1.30	p=0.55

Table 5.2.3 TNF alpha results from rising one year old deer from samples taken prior to and 5 minutes after intravenous injection of 0.5mg/kg of xylazine.

Animal	Pre sedation	During sedation
1	10.46	11.81
2	6.74	5.6
3	1.23	2.39
4	19.52	31.69
5	5.42	4.66
6	2.51	1.05
7	36.71	37.66
8	3.36	3.19
9	4.03	3.51
10	9.89	7.88
11	37.78	39.93
12	5.43	2.52
13	12.41	12.32
14	5.09	5.62
15	8.18	6.61
16	3.9	2.93
Mean	10.79	11.21
Standard deviation	11.26	12.99

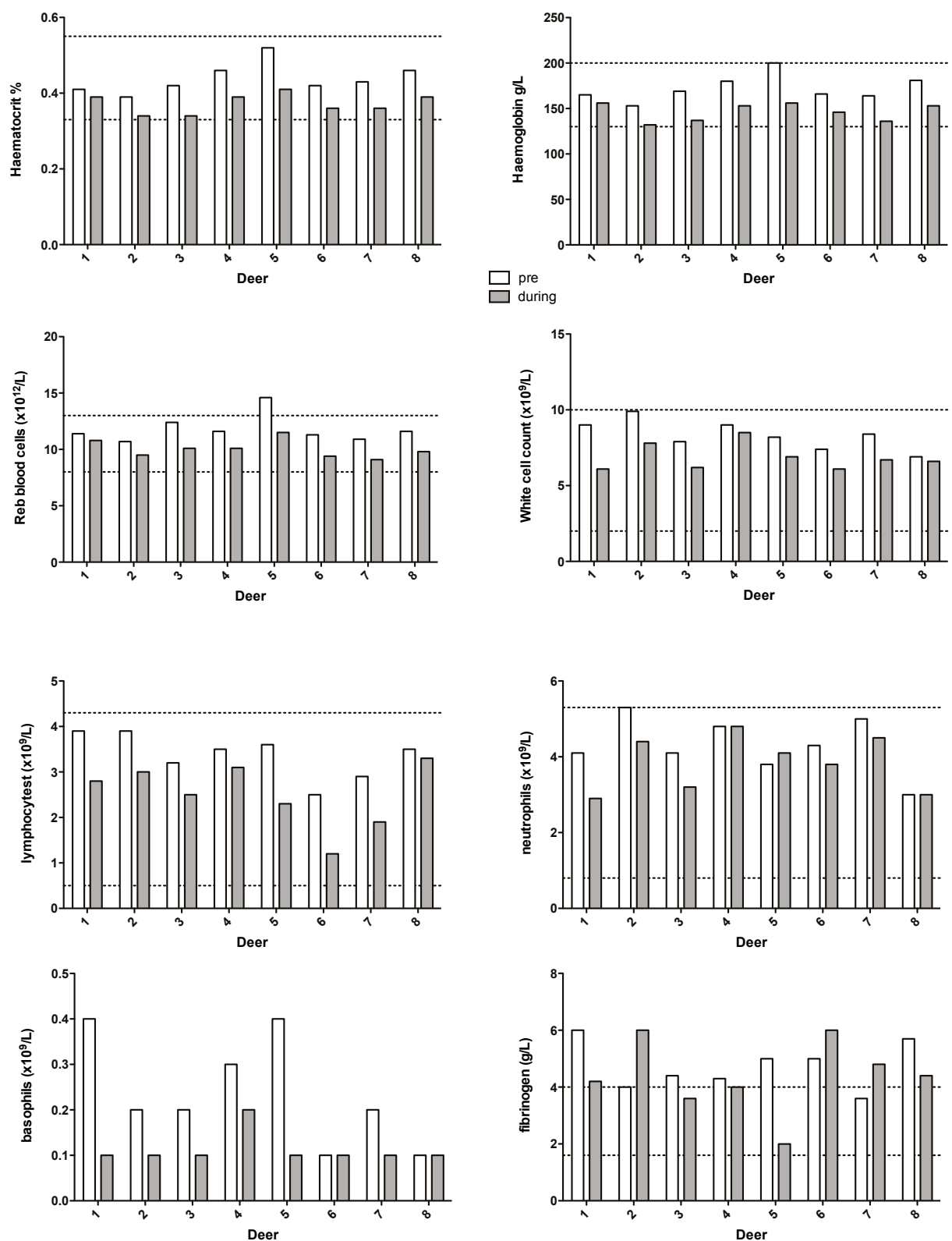


Figure 5.2.6 Haematology values from eight rising one-year-old deer before (pre) and 5 minutes after (during) sedation with intravenous xylazine. Dashed lines indicate upper and lower reference values from unsedated red deer from Cross et al (1989) and Wilson and Pauli (1982)

Discussion

This study aimed to investigate the direct effects of xylazine sedation on deer in order to gain a better understanding of the potential causes of post-xylazine deaths in stags. Deer that were sedated with xylazine in this study experienced changes in blood gas status, especially blood oxygen levels. Some animals had severe decreases in haemoglobin oxygen saturation as measured by pulse oximetry and/or arterial oxygen pressure as measured by blood gas analysis. In a veterinary clinical situation, the levels seen here would indicate a need to supplement with oxygen to avoid brain damage. These effects of xylazine may be related to the occurrence of post-xylazine stag death reported from commercial farms. There was marked variation between animals in their response to xylazine and this may reflect a variation between animals in their susceptibility to post-xylazine death.

There have been very few experiments published that examined the effects of xylazine in deer (without the addition of other anaesthetic agents) on blood oxygenation and this is the first report of the use of pulse oximetry immediately following intravenous injection of xylazine in yearling red deer. SpO₂ was often difficult to measure in the deer as a signal was not always detectable. Pre-sedation readings were often not obtained because movement of the animal caused the probe to dislodge. Pre-sedation recordings that were made were generally lower than would be expected in normal, awake animals although SpO₂ measurements have not previously been reported from deer without any sedation. Peripheral vasoconstriction due to the stress of handling may have been responsible for these readings. These levels are likely to be recording errors but do mean that caution should be exercised interpreting the readings that were obtained during sedation. In most deer, the SpO₂ was severely decreased after sedation, with 96% of animals dropping below 90% haemoglobin oxygen saturation at some point and 58/124 animals producing readings below 70% at which level the pulse oximeter is unlikely to be accurate. The lowest recording was 24% which indicates severe hypoxaemia likely to result in tissue damage if maintained for more than a few minutes. Readings were rarely made within the first one to two minutes because the probe could not detect a pulse. This is possibly due to vasoconstriction immediately after sedation.

There is very little published data of reference ranges of blood gas analyses of normal non-sedated deer. Bouda et al (2000) measured acid base parameters and electrolytes from venous blood in non-anaesthetised red deer weaners. Paterson et al (2009) recorded the mean and standard deviation of PaO₂ of 8 North American Elk hinds prior to anaesthesia and after insufflation of medical air as 100 ± 12 mmHg. This seems a reasonable range for normal PaO₂ and is similar to other species. These publications were used to estimate an expected normal range for our study but it would have been useful to take arterial blood samples from the deer in this study prior to sedation as well as during sedation so that direct comparisons could be made.

As for SpO₂, PaO₂ was low in all deer after sedation. Levels recorded here were similar to other studies that have used alpha 2 agonists in combination with other anaesthetic agents in wapiti (Caulkett et al, 2000; Paterson et al, 2009; Read et al, 2001) Chinese water deer (Bouts et al, 2011), White-tailed deer (Mich et al, 2008) Sika deer (Suzuki et al, 2001), Rocky Mountain Elk (Kree-

ger et al, 2011), Reindeer (Risling et al, 2011). Again, there are very limited data on the normal values of non-sedated deer.

This appears to be the first report of arterial blood gas analysis in red deer administered xylazine (or detomidine) alone. In most studies by other authors, deer that are not easily handled have been used and an opioid is usually included in the combination for immobilisation to improve the predictability of the response. High potency opioids are preferred when remote delivery of drugs is required so that a smaller volume can be used in the syringe. It is interesting that the PaO_2 values obtained in the current study are similar, albeit higher than the most extreme values, to other studies that have used combinations of alpha-2 agonists and opioids. As opioids have a respiratory depressant effect, it would be expected that the combination would produce a more severe hypoxaemia than xylazine alone. Rocky Mountain Elk that were immobilised with a combination of carfentanil and xylazine had mean SpO_2 of 23 ± 3.1 . After reversal with naloxone, this improved to 50.2 ± 7.3 (Moresco et al, 2001) indicating that the opioid had a significant additive effect on SpO_2 with the xylazine. Some of this may be due to reversal of the respiratory effects of the carfentanil or to lightening plane of anaesthesia. The lowest SpO_2 recorded in a North American Elk administered carfentanil + xylazine was 14mm Hg, a level at which severe tissue damage and death would be expected if prolonged. Detomidine did not cause as severe hypoxaemia or hypercapnia as xylazine. It is not certain why this would be the case. Some animals injected with detomidine were noted to be less well sedated than those injected with xylazine so it may have simply been due to a lighter plane of anaesthesia. There was no difference in the respiratory rate between the different drugs and heart rate tended to be higher in some deer that were sedated with xylazine than detomidine. If xylazine causes hypoxaemia by a peripheral alpha-2 receptor mechanism the difference may be due to receptor sensitivity to the different drugs in different cell types.

Caulkett et al (2000) point out that hypoxaemia is due to ventilation/perfusion mismatch or pulmonary shunting and suggest that this may be caused by hypoventilation. The Pa CO_2 levels were not increased to the same severity as the reduction in PaO_2 indicating that hypoventilation was only mild in the deer in this study. Oxygen supplementation either by nasal insufflation or endotracheal intubation increases PaO_2 (Read et al, 2001; Paterson et al, 2009; Mich et al, 2008). Supplementation with oxygen in Canadian wapiti deer anaesthetised with xylazine-tiletamine-zolazepam that were exhibiting a similar level of hypoxaemia to the deer in our study resulted in a marked improvement in PaO_2 indicating that hypoventilation is the main cause of the hypoxaemia and that this can be overcome with supplemental oxygen. Oxygen supplementation may result in more severe hypercapnia due to further respiratory depression. Six white-tailed deer sedated with a combination of butorphanol-azaperone-medetomidine (BAM) developed severe hypoxaemia that improved with oxygen supplementation and hypercapnia that worsened with oxygen supplementation (Mich et al, 2008).

This study is one of few studies where sedative agents have been injected intravenously and samples have been collected within 5 minutes of injection. It is likely that intravenous delivery causes a more severe respiratory depression in the initial stages due to a higher initial circulating concentration of the drug. When drugs are administered intramuscularly, and especially when darting is used to deliver the drugs, a minimum of 10 to 20 minutes has elapsed before sampling can take place. Paterson et al (2009) injected North American Elk in a crush via the intramuscular route and found that PaO_2 reached minimum levels at around 9 minutes after injection. In deer that

were not supplemented with oxygen, these levels remained low for the duration of the experiment so delayed sampling may not be a major problem in other studies when animals have been darted in pens. In practice stags that are sedated for velvet removal are invariably injected intramuscularly. Sedation of red deer hinds in New Zealand for embryo transfer is often done intravenously with “Fentazin” which is a combination of xylazine, azaperone and fentanyl citrate. This combination, administered by the intravenous route is likely to cause more severe hypoxaemia than intramuscular xylazine alone. Furthermore hinds are usually restrained in dorsal recumbency in the head down position and abdominally insufflated with carbon dioxide. It is recommended that in this situation supplementary oxygen be provided as a standard protocol, particularly when the viability of implanted embryos is important. Interestingly, no anecdotal or published reports of hind deaths following embryo transfer have been found. Providing oxygen during velvet removal from stags is not practical in a farming situation where farmers are administering sedative drugs to multiple stags themselves. The value of the harvested antler does not justify individual animal anaesthetics and oxygen supplementation. In most cases, only veterinarians would have access to the necessary equipment. As hypoxaemia is reversed, at least in the short term, by administering an α -2 antagonist, the most practical course is to complete the procedure quickly to minimise the time of hypoxaemia and to give reversal drugs at the completion of the procedure. If individual animals of high value could be identified, sedation with detomidine rather than xylazine along with nasal insufflation of portable oxygen may be a safer option.

pH and acid-base balance

pH was maintained within a narrow range. The mean pH was significantly higher in deer sedated with detomidine than xylazine and this is likely to be due to a correspondingly lower PaCO_2 in deer treated with detomidine. It is not clear whether the detomidine caused an elevation from normal pH or whether the xylazine caused a reduction. While the latter seems the most likely due to respiratory depression and elevation of PaCO_2 , the levels appear to be within the expected range for the xylazine group and above the expected range for the detomidine group. Bicarbonate values remained similar for both groups and within the expected range indicating limited renal compensatory effect although the time frame from drug injection to sampling may not have allowed for renal compensation. It is possible that normal pH levels are slightly higher in deer compared to other non-ruminant species but I have not been able to find any normal arterial pH values from non sedated red deer. There is limited data for venous pH. Paterson et al (2009) recorded a decline in pH over time from injection of carfentanil-xylazine combination in North American Elk. These developed acidosis ($\text{pH} < 7.3$) and this was worse for deer that were administered supplementary nasal oxygen compared to deer administered medical air.

Haematology

Walker and Middelberg (1988) reported increased eosinophils in the lungs of deer that died following sedation with xylazine and our study (Chapter 5.1) showed increased neutrophils in the lungs submitted for histopathology. Inflammatory cellular changes in the lungs may be reflected

in circulating leukocytes. Changes in circulating red and white blood cells (Cross et al, 1992) may be related to the mechanism of pulmonary pathology and post-xylazine stag death.

In general there was a decrease in the concentration of circulating red and white blood cells that was consistent in all eight deer sampled. The most pronounced change amongst the white blood cells was in lymphocytes which decreased in all deer by between 6 and 52%. All white cell types except monocytes and eosinophils decreased significantly after giving xylazine. Neutrophil counts were more variable and although the decrease in mean neutrophil count was statistically significant it is possible that this is a statistical aberration.

Decreases in red blood cell count and haemoglobin have been described (Cross et al, 1989) and are thought to be due to relaxation of the spleen under the influence of xylazine following splenic contraction during physical restraint when taking samples from unsedated deer (Wilson and Pauli, 1982). Cross et al (1989) showed that the fall in red blood cell count was greatly reduced in splenectomised deer compared to deer that had the spleen intact, supporting this theory.

Cross et al (1992) described changes in red and white cell counts in four 18-month red deer hinds. In that study the most dramatic change was a transient decrease in circulating basophils that recovered after 60 minutes. *In vitro* addition of xylazine solution to whole blood resulted in morphological disruption of basophils. The authors suggest that this may be a factor in post xylazine stag death but do not speculate as to the mechanism. Release of histamine from disrupted local basophils may contribute to pulmonary oedema. Basophils decreased by up to 75% in 2/8 deer in the current study at 5 minutes after intravenous injection of xylazine. Other deer had low basophil counts pre sedation and the reporting range from the laboratory is likely to have been a limiting factor in the precision of estimating changes in these animals. There did not appear to be a relationship between the drop in basophils (or any other blood cell type) and the SpO₂ recorded during sedation of these deer. The two animals with the greatest changes in basophils (1 and 5, Figure 5.2.6) had mean SpO₂ of 84.5 and 84 respectively during sedation which was relatively high compared to other animals. Deer 3 had an average SpO₂ of 60% and a minimum of 49% with moderate changes in white cell values and deer 8 had a mean SpO₂ of 70% and a minimum of 65% and showed very little change in any of the different white blood cells after sedation.

Other studies have also reported decreases in lymphocyte counts following administration of xylazine and this appears to be a more prolonged effect than the transient change in basophils. Cross et al (1992) found that lymphocyte numbers were still substantially reduced 60 minutes after giving xylazine. These authors also found that between 5 and 10 minutes after adding xylazine to whole blood, some morphological changes were evident in the lymphocytes possibly indicating imminent cellular disruption. It is still unclear whether the lymphocytes are being removed from the circulation by sequestration in tissue or by cellular disruption. Cross et al (1989) showed that reduction in lymphocytes occurred to a similar extent in splenectomised deer as deer with the spleen intact, indicating that splenic sequestration is not responsible for the decrease in circulating lymphocytes.

TNF alpha

TNF alpha was analysed from samples taken prior to and 5 minutes after injection of xylazine

to investigate for evidence an acute inflammatory response. The test had been newly established by a PhD student at Otago University using bovine antibodies. It appeared to be effective in quantifying deer TNF alpha. There were no consistent changes between the two sampling times for individual deer and there was no statistical difference in mean values. The variation between animals was large but repeated sampling on the same animal yielded consistent results. The time frame for sampling in the current study may have been too short to detect a significant change in this cellular mediator and repeated sampling up to 48 hours after sedation would have been of interest. If TNF alpha is to be used in the future, it will be important to use the same animals as controls because of the large variation between animals and also to carefully consider the time period for expected changes or to perform repeated sampling. The testing was relatively expensive which may provide limitation to the number of samples that can be taken.

Haptoglobin

Four serum samples that were still in storage were sent to Otago University for haptoglobin testing because haptoglobin has been identified as an acute phase inflammatory protein in deer (Cross, 1988). Reference values for haptoglobin in deer have subsequently been developed for adult hinds (Cross et al, 1994) and these range from 0.02 to 0.18. The authors of that study (J. Cross) suggested that values above 0.3 are abnormal and above 1.0 indicates a severe tissue damage (Cross pers. comm.). The results from our samples were all above 0.18 and two were well above 1.0 indicating an inflammatory condition in these animals. It is most likely that such a condition was present before sedation as the time frame was too short between injection of xylazine and blood sampling to expect a significant rise in haptoglobin. Mackintosh and Cross (1989) found a significant rise in haptoglobin 48 hours after deer were sedated with xylazine and Cross et al (1988) identified a rise in haptoglobin 72 hours after skin testing for tuberculosis. While the results in this study did not shed much light on the mechanism of post xylazine stag death, haptoglobin is worth including in xylazine studies in the future and it is relatively less expensive to test for compared to other acute inflammatory mediators. The high haptoglobin results in these four animals also indicates that an inflammatory process was likely to be occurring but it was not detected by clinical observations or changes in fibrinogen or white blood cell counts prior to sedation.

Conclusions

Intravenous xylazine and detomidine cause hypoxaemia in all red deer when administered alone. This can be determined by pulse oximetry and arterial blood gas analysis. Changes occurred rapidly (within 5 minutes) and were more severe with xylazine than detomidine. Hypoxaemia is accompanied by mild hypercapnia and does not appear to be fully explained by respiratory depression.

Intravenous xylazine caused decreases in red cell count, haemoglobin, white cell count, lymphocytes, basophils and neutrophils but not monocytes or eosinophils, fibrinogen or TNF alpha. Cellular changes did not appear to be correlated to the level of hypoxaemia.

5.3 Observation of yearling stags sedated with “Fentazin” (fentanyl-azaperone-xylazine combination) and given oxygen during sedation

Introduction

This clinical observation was made during sedation of stags that were being sent to Massey University from a Landcorp farm as part of a breeding value evaluation programme measuring meat to bone ratio using the computed tomography equipment and expertise at the Massey University Veterinary Teaching Hospital. Data were collected during sedation to compliment the data from Chapter 5.2.

Materials and Methods

Twenty six-month-old male wapiti deer were sent to the Massey University Veterinary Hospital for Computed Tomography scanning to determine meat to bone ratio (Figure 5.3.1).

Each stag was heavily sedated with an intravenous injection of 1mL of “Fentazin 5” (Bomac Laboratories Ltd, New Zealand). This provided 58.3mg of xylazine, 3.2mg of azaperone and 0.4mg of fentanyl citrate per stag. The animals weighed (mean $\pm$ SD) 87.6 ± 4.2 kg. This equated to a dose rate of 0.67 ± 0.03 mg/kg xylazine, 0.037 ± 0.002 mg/kg of azaperone and 4.58 ± 0.21 μ g/kg fentanyl. After the stag became recumbent it was strapped into a specially designed cradle to hold it in position for the CT scanner.

A catheter was placed in the marginal ear vein and the stag’s trachea was intubated using a size 14 ID endotracheal tube. A pulse oximeter (Nellcor N65) was placed on the tongue to record hae-



Figure 5.3.1 Yearling stag sedated with fentazin and strapped into a specially designed cradle for CT scanning of meat to bone ratio. The stag is connected to an anaesthetic machine supplying oxygen being monitored for oxyhaemoglobin saturation using a pulse oximeter

moglobin oxygen saturation. The stag was moved to the CT table and connected to an oxygen supply via an anaesthetic machine and allowed to breathe spontaneously. At least one pulse oximeter reading was made prior to oxygenation and at least one reading was made after the stag had been supplemented with oxygen for approximately 5 minutes.

After scanning was finished stags were injected intravenously with 1.5mL of Contran H (Bomac Laboratories Ltd, New Zealand) giving 15mg of yohimbine and 0.15mg of naloxone. Deer were monitored until recovered. When head and neck movements were evident, the endotracheal tube was removed.

Results

Table 5.3.1 and Figure 5.3.2 show pulse oximeter readings after sedation with xylazine before and during CT scanning with and without oxygen supplementation. All 20 stags had pulse oximeter

<i>Table 5.3.1 Pulse oximeter readings from yearling stags that were sedated with Fentanyl for CT scanning and provided with supplemental oxygen during CT. Readings after sedation and before starting oxygen (pre CT) and during CT with and without oxygen are shown.</i>				
Deer Tag	Pre CT No oxygen	During CT With oxygen	During CT No oxygen	Comments
186	49, 59	98		
158	76	86		
163	74		77	Not intubated
129	65	97		
162	68			No reading during CT
157	72	94		
230	36, 33		35	Not intubated Took a long time to recover
246	79, 69	92		
222	59	96		
249	50, 57			No reading during CT
218	88, 82	92, 90		
247	44	78		
240	87		84	Not intubated
212	67	92		
176	44, 32, 28	76, 88		
229	63	88		
223	67	95		
150	48, 45	95		
200	48	88		
214	96, 89		68	Not intubated Getting up on table
Mean	63.35	90.86	66	
Range	28-89	76-98	35-84	
SD	16.4	5.9	21.7	

readings prior to oxygen supplementation. 4 stags were unable to be intubated successfully and were not supplemented with oxygen. Two stags did not produce readings on the pulse oximeter during the CT scanning period.

After sedation with “Fentazin” and prior to oxygenation the pulse oximeter reading ranged from 28% to 89%. The mean was 63% and the standard deviation was 16.4%. After oxygenation the mean increased to 91% (range of 76 to 98%; standard deviation 5.9). This increase was significantly different from pre-oxygenation recordings with a mean difference of 27.5% and t-test result $p < 0.0001$

For the four deer that were not able to be intubated the mean was 66% with a range of 35 to 84% and a standard deviation of 21.7%. This was not significantly different from pre-CT recordings. ($p = 0.78$).

One stag that was unable to be intubated and had a pulse oximeter reading of 35% during CT scanning took a long time (>10 minutes) to recover after injection of the reversal agent. The stag recovered fully and was returned to the farm of origin.

Discussion

During the pre-oxygenation period all stags had readings below 90% which is consistent with the recordings in Chapter 5.2 and is considered a level requiring oxygen supplementation. Some readings were especially low with readings of 50% or lower in 8/20 animals. At this level, the

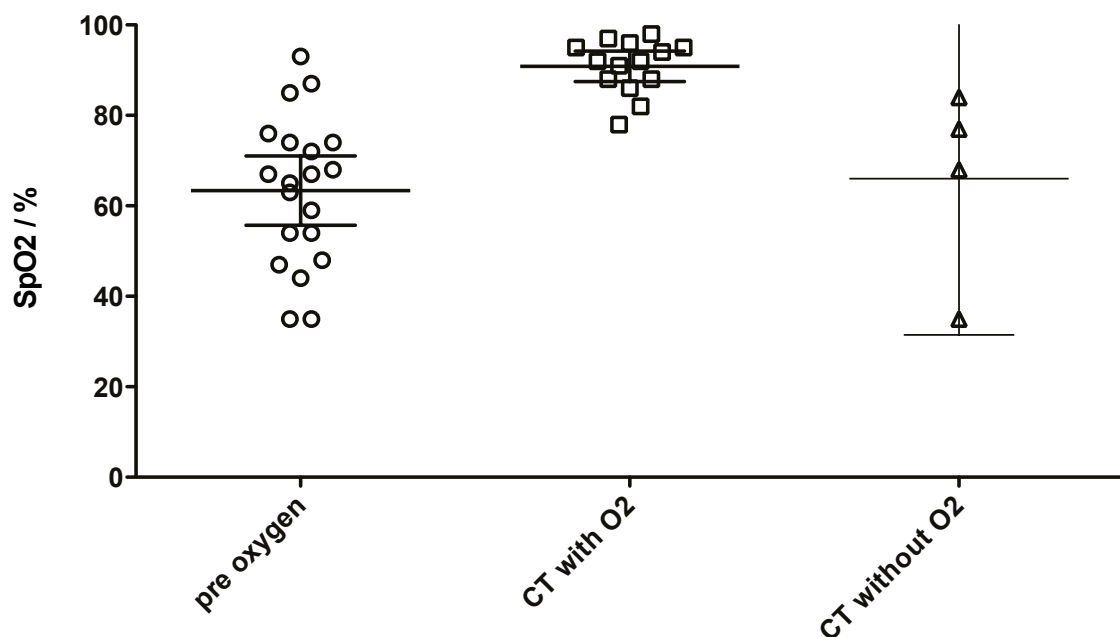


Figure 5.3.2 SpO2 readings from a pulse oximeter showing haemoglobin oxygen saturation levels of stags that had been sedated with Fentazin for CT scanning and either supplemented with oxygen or not supplemented with oxygen during CT. Mean and 95% CI of the mean are shown.

accuracy of the pulse oximeter is likely to be reduced but it was well below acceptable levels and indicated severe compromise of the respiratory system in the ability to transfer oxygen from the air into the blood.

After oxygenation, the increase in SpO₂ was significant with an average increase of 22%, in contrast to the four deer that were not able to be intubated which all maintained approximately similar SpO₂ readings throughout except deer 214 which decreased during the CT period.

The increase in SpO₂ readings after supplementation with oxygen is encouraging and consistent with other studies that have shown a marked increase in PaO₂ with oxygen supplementation (Patterson et al, 2009). The results also give weight to the argument that all deer should be supplemented with oxygen during sedation with alpha-2 agonist agents (Read et al, 2001), particularly when used in combination with opioid drugs. The easiest way to achieve this in the field is likely to be intranasal flow-by oxygen as described by Read et al (2001). The increase in SpO₂ in response to oxygen supplementation suggests that the lung tissue is still able to exchange oxygen to the blood even in animals that initially have very low SpO₂ levels provided the alveolar oxygen tension is great enough. If pulmonary shunting due to a physical barrier in the lungs in the form of oedema occurs in response to xylazine then this is able to be overcome by increasing the oxygen partial pressure within the alveoli. This was achieved when deer were allowed to breathe spontaneously as personnel were not able to enter the CT room to provide assisted ventilation.

All of the stags in this observational study recovered fully. While these results give some indication as to the effect of the xylazine-fentanyl-azaperone combination on the pulmonary tissue they do not tell us whether this is the cause of death in deer that have died after sedation with xylazine.

It appears that yearling wapiti stags have large individual variation in their pulmonary response and SpO₂ readings after intravenous Fentazin. There may be a breed difference and wapiti may be more or less susceptible to the effects of the drug compared to red deer. The results of the current study do not support a breed effect, although it is difficult to compare these results with those from experiment 5.2 because the combination drug “Fentazin” was used here rather than straight xylazine as was the case in experiment 5.2.

Conclusions

“Fentazin” caused decreases in haemoglobin oxygen saturation in yearling deer to a level comparable with other research.

Supplementation with oxygen increased haemoglobin oxygen saturation and should be considered in all deer sedated with “Fentazin”.