The effect of predator presence on the behaviour of sheep in pain

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Abstract

In the veterinary and ecology literature two claims regarding predator-prey interactions are frequently made. Firstly, that vertebrate predators typically capture disadvantaged individuals, including the young, weak, sick, aged and injured, from prey populations in higher than expected proportions. Secondly, as a consequence of this, prey animals when injured or diseased, are said to attempt to behave in a normal manner, similar to that of a healthy conspecific, so as not to draw the attention of a predator.
This thesis investigates whether the presence of a predator modifies the behaviour of sheep and lambs that are in pain.

There are two parts to this thesis. Part one examines the behaviour of lambs following castration. Part two examines the behaviour of adult sheep in response to a mechanical pressure device.

The aim of part one was to monitor the restlessness behaviour of lambs following castration in the presence of four stimuli (a goat, a dog, a cardboard box, and a tape recorder playing the sound of a dog barking), and the effect that the lambs' dam has on this behaviour. Three hundred and eighty four lambs were observed in this study, half of these lambs were castrated and the other half were left as controls.

Castrated lambs exhibited more restlessness behaviour than control lambs ($p<0.0001$). However, restlessness behaviour was not different between lambs exposed to one of the four stimuli. The presence of the lambs' dam significantly reduced the restlessness behaviours of both castrated ($p<0.0003$) and control lambs ($p<0.0019$).

The aim of part two was to determine the threshold response of 16 adult Romney ewes in response to a painful mechanical pressure device in the presence of four stimuli (a dog, a goat, a tape recording of a drum beat and a tape recording of a dog barking).

The response threshold was higher in sheep exposed to the dog than to the goat ($p<0.05$).

The significant difference between the behaviour of castrated and control lambs suggests that castration is a painful procedure. Moreover, lambs may rely on their mother to cue them on how to behave, as lambs significantly reduced the amount of restlessness behaviour they exhibited when their ewe was present (castrated lambs $p<0.0003$; control lambs $p<0.0019$). Adult sheep find dogs aversive, and their pain thresholds were higher in their presence than when a goat was present. This suggests that sheep are able to alter their behaviour in the presence of a potential threat.

These results justify further research into the behaviour of prey animals in the presence of a predator. Two key avenues for future research include; determining how prey animals view humans, and further investigating the mother-young relationship and the affect the presence of a predator has on this.

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I dedicate this thesis to my husband Bevan, and Jess, because with their unfailing optimism, understanding, companionship and support, I feel I can achieve everything and anything.

The use of all animals and procedures in the experiments described in this thesis were approved by the Massey University Animal Ethics Committee. Reference Numbers 03/122 and 03/11 (Appendix 7.3). In addition, it is intended that Chapters 3 and 4 of this thesis will be published as two papers in scientific journals. The authors of these papers will be Suzanne Young, Kevin Stafford and Ed Minot.

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1 General Introduction

Sheep and goats were domesticated more than 8,000 years ago in Western Asia (Rutter, 2002; Clutton-Brock, 1999). The domestic sheep rapidly spread into Europe and north and east into the rest of Asia and the Far East (Clutton-Brock, 1999). Domestic sheep (*Ovis aries*) are believed to be derived from the Asiatic Mouflon (*Ovis orientalis)*, which lives in dry and mountainous regions of Asia (Rutter, 2002). By the sixth millennium BC, large phenotypic changes had occurred in sheep. Female sheep became horn-less, and their fleece had changed from the wild type to an entirely white, woolly fleece (Clutton-Brock, 1999).

Selective breeding over many years has resulted in calmer, more productive, and heavier sheep (Vangen et al., 1994, cited in Hansen, Christiansen, Hansen, Braastad, & Bakken, 2001), and it is possible that the process of domestication and the reduced risk of predation have reduced anti-predator behaviours in sheep (Eggen, 1995, cited in Hansen et al., 2001).

In the veterinary and ecology literature it is regularly proposed that prey species, when injured or diseased, will attempt to behave in a normal manner, similar to conspecifics, so as not to draw the attention of a predator (Short, 1998; Taylor, Pascoe, & Mama, 2002; Underwood, 2002). This hypothesis has not been tested.

Behaviour is used to assess the stress animals experience when exposed to a threat (Dwyer, 2004). However, the measurement of behaviour is difficult and the interpretation of behaviour is often subjective (Mason & Mendl, 1993). Behavioural responses to stress are thought to derive from the anti-predator responses of a species (Blanchard et al., 1998, cited in Dwyer; Boissy, 1998, cited in Dwyer, 2004). Frid and Dill (2002), suggested that prey species have developed anti-predator responses to predators and to threatening stimuli, such as loud noises or sudden events. This supports the concept that stress responses are anti-predatory behaviours in prey species. Thus, understanding the functional significance of anti-predatory behaviours may help in the interpretation of behavioural responses to stress (Dwyer, 2004). This may encompass behavioural responses to painful stimuli as well.

Prey animals may change the pain-related behaviours they exhibit in response to the environment they are in. Because behaviour is the most commonly used parameter for assessing animal pain, the possibility that animals may alter their pain related behaviour may make the assessment of pain using behaviour more difficult. For example, a lack of overt behaviour indicative of pain in some farm animals after husbandry procedures such as castration has been interpreted to indicate that the animal is not experiencing pain. The human observer may be perceived as a predator and this may affect the animal's behaviour.

The sheep was chosen as the model for this thesis because the literature on the behaviour of wild and domestic sheep is particularly rich. In addition, the behaviour of lambs following castration and tail docking has been extensively studied over the last twenty years (Mellor & Stafford, 2000). Castration is a painful procedure, and a set of behaviours and postures have been identified as indicators of pain in lambs. Therefore the sheep is a good model for studying pain related behaviour in the presence of a predator.

In this thesis the effect of the presence of a predator on the behaviour of sheep in pain will be examined.

In Chapter 2 the literature regarding the methods used to evaluate pain in animals is reviewed, as is the response of prey animals and especially sheep to predators and other stimuli.

Chapter 3 describes the behaviour of lambs following castration when they are held in pens in the presence of four different stimuli; a dog, a goat, a cardboard box and the sound of a dog barking.

In Chapter 4 the response of ewes to a painful mechanical pressure device is described in relation to the presence of four different stimuli (dog, goat, tape recorder playing the sound of a drum beat and a tape recorder playing the sound of a dog barking).

Chapter 5 is a general discussion of the results of the research and their significance is evaluated. The future direction of this type of research is discussed.

Chapter 6 contains the list of references used in this thesis.

Chapter 7 contains appendices with the data from the experimental components of this thesis.

2 Literature Review

Pain has been defined as "an unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP, 1979, p.250). In the past, there has been debate over whether animals feel pain.

Over the past 15 years, significant advances have been made in our understanding of pain in animals. The debate as to whether animals possess a similar capacity as man to experience pain has been debated for centuries (Flecknell, 2000). The debate continues because it is impossible to study the experience of pain directly. Solipsism states that the only consciousness a person knows is their own (Bateson, 1991), and consequently, before pain can be recognised and alleviated in animals, this philosophical perspective must be rejected. Even though it may be impossible to study pain in animals directly, inferences about it can be drawn from indirect measures, such as behaviour.

2.1 Animals and Pain

There is evidence that animals experience physical pain in a similar manner to humans (Morton, 2001), and a range of tests, have demonstrated animals' ability to detect damaging or potentially damaging stimuli, for example excess heat or mechanical pressure (Broom, 2001). These tests include the tail flick response, the jaw opening response and limb withdrawal (Broom, 2001). The possession of a detection system does not, in itself, mean that animals experience pain. Pain in humans is defined as having both a sensory and an emotional component. If the brain does not interpret the sensory information arriving from peripheral nerves, the characteristic unpleasant and distressing nature of pain would not be experienced (Flecknell, 2000).

The initial detection of tissue damage is by a peripheral detection system, termed 'nociception'. Nociception is the electrical or chemical activity of nociceptors. The perception and interpretation of this information centrally results in the experience of pain. Animals and humans possess nociceptors, and the types of nerve fibres that connect them to the central nervous system, are virtually identical in all animals (Flecknell, 2000). For example, pain and pleasure centres in the brains of birds, mammals, and fish are very similar to those in humans (Rollin, 2003). In addition, the neural mechanisms responsible for pain behaviour are similar in all vertebrates, and the presence of endogenous opiates in animals, suggests that they feel pain (Rollin, 2003). It has been argued that there would be no reason for animals to have similar neurochemicals and pain inhibiting chemicals to humans if they do not experience pain in a similar manner to humans (Rollin, 2003). It is presumed by many that the presence in animals of those physiological characteristics which in humans are known to cause pain, will cause pain and suffering in animals as well (Wall, 1991; Short, 1998)

However, it is important to note, the experience associated with pain may not necessarily be the same for both humans and animals. What one animal experiences when it is in pain may be very different from that of another animal of the same species. In addition, the signals sent to the brain informing of the damage, are processed differently between species (Appleby, 1999). Major differences may arise when the information reaches the cerebral cortex. Because of these differences, it is essential that animal-based studies are performed. Some animal pain may be overlooked, due to an over-reliance on comparisons between human and animal pain (Flecknell & Molony, 1997).

It has therefore been established that animals, by inference, feel pain.

2.1.1 The function of pain

The way in which pain is perceived can be modified by previous experience, emotional state and perhaps innate individual differences. Pain is of evolutionary value. The ability to feel pain is essential for human survival (Rollin, 2003), and it is therefore assumed that pain is felt in animals for the same reasons. Pain serves to warn an animal of tissue damage, to elicit immediate escape, withdrawal or recuperation behaviours and it creates the opportunity to learn to avoid similar pain causing circumstances in future. Thus it has survival value, serving to increase fitness.

The physiological state and behaviour of an animal can be altered by pain as it attempts to reduce or avoid damage, and reduce the likelihood of recurrence and aid recovery (Molony & Kent, 1997). The unpleasant component of pain may serve as an integral part of its evolutionary function (Dawkins, 1998). Chronic pain, however, has often been referred to as non-functional pain, as it remains long after it can serve any useful purpose (Melzack & Wall, 1996; Molony, 1997, cited in Rutherford, 2002).

2.1.2 Assessing pain

An animal's behaviour may indicate pain and the alleviation of pain. When left untreated, pain can result in weight loss, muscle degeneration, impaired respiratory function, increased blood pressure and the reduction of self-maintenance behaviours (Flecknell, 2000). Failure to manage pain can lead an animal to self mutilate and/or result in chronic pain.

The direct measurement of a subjective experience such as pain is not possible, and so the assessment of animal pain is based on physiological and behavioural responses (Molony & Kent, 1997). The absence of normal behaviour is the most striking sign that an animal is in pain (Anil, Anil, & Deen, 2002). Animals convey their pain experience by altered behaviours and by physiologic changes. Methods for assessing animal pain include measuring, restlessness, lameness, self-mutilation and vocalisation, as well as changes in eye expressions, appetite, temperament, and physical activity (Short, 1998).

Physiological responses have been used to assess pain. These include respiratory rate, heart rate, blood pressure, food and water consumption, and endocrine responses such as catecholamine or adrenocortical hormone responses. These variables may all be of value in assessing the degree of pain experienced by an animal, but they are of limited use as predictors of pain severity when used alone (Conzemius, Hill, Sammarco, & Perkowksi, 1997; Hansen, Hardie, & Carroll, 1997; Molony & Kent, 1997; Holton, Scott, Nolan, Reid, & Welsh, 1998). They can be influenced by fear and anxiety (Flecknell, 2001) as well as exercise (Anil et al., 2002). In addition, some of these variables may also be influenced by analgesic drugs (Flecknell, 2001). However, physiological variables are useful, and combining physiological and behavioural measures can be very useful.

One problem with measuring animal pain is validity. Validity is defined as the extent to which a scale actually measures what it is intended to measure (Streiner & Norman, 1989). Validity is important because a scale can be reliable and sensitive without actually measuring what it is intended to measure. When an established scale exists, a new parameter can be validated against it. In the case of animal pain, however, there are no established objective measurement scales against which to validate any new scale. This raises the question of whether measurements of animal pain can ever be truly validated. In humans, physiological and behavioural scales can be validated against verbal self-report of pain. Animals' lack this ability, but certain methods have been developed for humans to whom verbal report is not reliable or possible (Streiner & Norman, 1989). Examining a correlation between several independent measures can provide an alternative source of validation. The collection of both physiological and behavioural data to measure the same variable can be used as an alternative method of validation for identifying animal pain. For example, the patterns of cortisol release in lambs following castration and tail docking correspond closely with behavioural responses (Kent, Molony, & Robertson, 1993; Molony, Kent, & Robertson, 1993).

Because there are difficulties associated with the measurement of physiologic indicators of pain, observation of behaviour remains the most common and the most useful method for recognising pain in animals (Hansen, 1997; Livingston & Chambers, 2000; Rutherford, 2002). Using behaviour to assess pain has the major advantage of being non-invasive, so any effects of assessment on the animal are limited. Detailed objective assessments of behaviour have been used to assess pain in laboratory (Liles & Flecknell, 1991), companion (Hardie et al., 1997) and farm animals (Molony et al., 1993; Molony, Kent, Hosie, & Graham, 1997; Molony, Kent, & McKendrick, 2002).

Various approaches have been used to assess animal behaviour in relation to pain. These include the use of pain scales, motivation tests, observations of behaviour following treatment, individual and species specific responses to pain, and using analgesia and anaesthesia.

2.1.3 Pain scales

Clinical evaluation of animal behaviour is often subjective, and different observers watching the same animal may reach markedly different conclusions. To reduce this inter-observer variability, several attempts have been made to evaluate pain more objectively by using pain-rating scales based on observation. These rating scales have been modelled on pain-rating scales developed to assess human pain. These methods of scoring animal pain have been restricted to the use of three one-dimensional rating scales; the simple descriptive scale (SDS), the numerical rating scale (NRS) and the visual analogue scale (VAS). All of these really measure only one aspect of the pain experience, namely intensity. However, pain is a multidimensional experience, and the use of one-dimensional scales is limited. Moreover, these scales have been shown to be unreliable in the veterinary setting (Holton et al., 1998), and a poor association was found between an NRS and physiological data in a study on postoperative pain in dogs (Conzemius et al., 1997). Attempts have been made to construct multidimensional scales to measure pain in animals, but although these scales define behaviours which are thought to be indicative of an animal in pain, they do not fulfil the criteria of a valid, reliable multidimensional or composite measure scale for use in animals for the purpose of rating pain (Morton & Griffiths, 1985; Sanford, Ewbank, Molony, Tavernor, & Uvarov, 1986; Dodman, Clark, Court, Fikes, & Boudrieau, 1992; Firth & Haldane, 1999). All of these pain-scoring techniques rely primarily on human observations of behavioural signs, and interpretation of these is highly subjective (Dobromylskyj et al., 2000).

Successful development of any pain scale requires detailed knowledge of the pain behavioural patterns of the species for which the tool is designed, and scales cannot be transposed from one species to another.

2.1.4 Motivation

Animals can be trained to perform complex tasks to avoid brief painful stimuli such as electric shocks, and to self-administer analgesics when they develop chronic painful conditions. When an animal responds to a painful stimulus the magnitude of the response may give some indication of the level of pain the animal is experiencing. It is assumed that extreme responses, which place a significant energetic cost on the animal, are associated with severe pain (Rutherford, 2002). Extreme behavioural and physiological responses may use up much of an animal's time, and substantial bodily resources respectively (Rutherford, 2002). In some studies of pain, operant methods have been used to measure the motivation of an animal to escape or avoid a painful stimulus (Chapman et al., 1985). It is presumed that the greater the pain, the harder an animal will work to avoid it.

The presence of pain may be inferred with even more certainty if an animal, subject to a painful stimulus, is given the opportunity to administer an analgesic to itself and does so (Colpaert, 1987). Danbury, Chambers, Weeks, Waterman, and Kestin (1997) and Danbury, Weeks, Chambers, Waterman-Pearson, and Kestin (2000) have used analgesic self-administration experiments to assess pain due to lameness in broiler chickens. In these experiments, lame broilers self-selected both morphine and the non-steroidal anti-inflammatory drug (NSAID) carprofen. However, healthy birds also showed a preference for morphine, so care must be taken in such experiments to ensure that the drug is selected for its analgesic properties, and not for other reasons (Rutherford, 2002). Similarly, experiments that allow animals the opportunity to self-select an analgesic (Colpaert, 1987; Danbury et al., 2000) may be adapted to test the motivation of an animal to gain pain relief (Rutherford, 2002).

2.1.5 Behaviour following treatment

It is possible that behaviour may indicate pain, if it is exhibited by animals subjected to painful procedures during or after such procedures but not by control animals (Dinniss, Stafford, Mellor, Bruce, & Ward, 1999). There are many illustrations of this. The behaviour of lambs following castration and tail docking is a common example (Lester, Mellor, Holmes, Ward, & Stafford, 1996; Thornton & Waterman-Pearson, 1999). Lamb behaviour following castration and tail docking was first quantified by Mellor and Murray (1989). They found that castrated and docked lambs exhibited a greater incidence of abnormal postures, such as ventral lying with partial or full hind-leg extension, and restlessness than control animals.

2.1.6 Individual and species specific responses to pain.

Behaviour associated with pain may be specific to a species, and to specific types of tissue damage (Lester et al., 1996; Dinniss et al., 1999). It is important in the diagnosis of pain in animals to appreciate the species, breed and individual differences in their response to injury (Short, 1998). Knowledge of the selective pressures on different species is essential if behavioural responses are to be properly interpreted (Broom, 2001). It is likely that behavioural pain responses are adaptive, and this may determine whether an animal demonstrates overt pain related behaviour or not. When some species are injured, it may be beneficial for individuals to vocalise that they are in pain (Fraser & Broom, 1997), whereas, herbivores such as antelopes may hide their pain in the presence of a predator so as not to alert predators of their reduced fitness (Sanford et al., 1986; Anil et al., 2002). In addition, herd animals such as herbivores become stressed when isolated from their peers, and thus, may alter their expression of pain behaviours (Dombromylskyj et al., 2000).

Animals may adjust their behaviour according to their surroundings, such as masking pain-related behaviour in unfamiliar environments. Different injuries may elicit unique behavioural responses because the sensations experienced by an animal may differ when different tissues are injured or similar tissues are damaged in different ways. For instance surgical castration and/or tail docking of lambs cause immobility whereas ring castration and/or tail docking cause increased activity (Molony et al., 1993; Lester et al., 1996; Dinniss et al., 1999). In addition, different characteristic postures can occur in response to pain in different areas of the body (Hansen, 1997). This specificity of behaviour means that detailed study of animals following particular procedures is necessary to identify general and specific behavioural responses to different types of tissue damage and pain.

Furthermore, there are inter-individual differences in the experience of pain. These differences can be attributed to genetic factors (Mogil, 1999) such as differences between species and between breeds in the case of the dog (Reid, 2003), or the individual's early experience and environment (Rutherford, 2002). Other causes of these differences include age, sex, reproductive status, group size, cage or pen size, food and water intake and the environment (Anil et al., 2002).

The unique behaviour evoked by specific injuries makes it difficult to use behaviour alone to compare the pain experienced by animals subjected to different treatments (Mellor & Holmes, 1999). Because behavioural responses to different treatments are injury specific, it is necessary to monitor physiologic distress responses to assess the relative effects of such treatments. In addition, if behaviour is to be used to compare the pain experienced by animals, single behaviours need to be consistently expressed in response to different treatments (Lester et al., 1996).

2.1.7 Analgesics and anaesthesia

Analgesics and anaesthetics are useful tools to use in the assessment of pain. Pain is a perception that depends on activation of a discrete set of nociceptors by noxious stimuli. However, an additional characteristic of nociception is that it is possible for nociception to occur without the conscious perception or awareness of pain if nociceptor nerve impulses are blocked peripherally or where they enter the spinal cord or in the brain before they reach the higher centres. By administering analgesics or anaesthetics, it is possible to block the nociceptor response from travelling to the brain. Using this knowledge, it is possible to use behaviour to identify nociception and by inference, pain, if effective local or regional anaesthesia or systemic analgesics return behaviour levels close to those exhibited by control animals (Dinniss et al., 1999).

When assessing pain, it is necessary to combine analgesic treatment with the measurement of behavioural changes. If an animal is showing abnormal behaviour, and this abnormal response is returned to 'normal' by the administration of an analgesic, the inference is made that the abnormal behaviour was caused by pain.

This method of pain assessment and behaviour identification has been used extensively in relation to the behaviours exhibited by castrated and tail docked lambs. Wood, Molony, Fleetwood-Walker, Hodgson and Mellor (1991) found that lambs given local anaesthetic prior to castration and tail docking did not exhibit the behavioural and endocrine changes normally seen. In addition, Thornton & Waterman-Pearson (1999) were able to distinguish between behaviour associated with the pain at the time of castration and post castration pain, by administering general anaesthesia during castration. In both of these studies, control groups that underwent handling but no other procedure were also included, and treatment animals, which were given anaesthesia only, were found to behave in a manner similar to those lambs which were only subjected to handling (Wood et al., 1991; Thornton & Waterman-Pearson, 1999).

There is danger that using the effects of analgesic treatment on behaviour to recognise pain becomes a circular argument. For example, pain is something removed by an analgesic; and an analgesic is something that removes pain (Bateson, 1991). Ideally, the presence of pain should be validated with other indicators. It should also be noted that analgesic drugs may have behavioural effects unrelated to pain and nociception. For instance, some analgesic drugs also have general sedative effects, which decrease activity (Hardie et al., 1997). It is therefore important in experiments, to have a control group that only receives analgesic treatment, to establish the effect(s) this has on animal behaviour.

2.2 Effects Of Predators On The Behaviour Of Prey Species

"Any property of an organism that reduces its chances of being taken by a predator will be favoured by natural selection" (Ridley, 1995, p. 152).

In the veterinary and ecology literature, two claims regarding predator-prey interactions are frequently made: Firstly, that vertebrate predators typically capture disadvantaged individuals, including the young, weak, sick, aged and injured, from prey populations in higher than expected proportions. Secondly, as a consequence of this, prey animals when injured or diseased are said to attempt to behave in a normal manner, for instance similar to that of a healthy conspecific, so as not to draw the attention of a predator.

If these statements are true, then we should see evidence that predators are selecting disadvantaged prey over healthier animals. From a behavioural ecology perspective this prediction coincides with optimal foraging theory, whereby animals should exploit a food source that provides maximum energy as a result of exerting minimal energy. If prey are disadvantaged, it is probable they would be slower and easier to catch than their healthier conspecifics, and as a consequence, more susceptible to predation. However, the relative benefits of capturing substandard prey should be greatest when a predator is targeting a species that is typically difficult to capture and kill (Temple, 1987).

Furthermore, prey species should be under selection pressure to hide any signal that a predator might use to detect compromised escape ability. Thus an injured animal may conceal any behaviour that signals it is in pain. If predators do select disadvantaged prey, these predators must be using specific methods to detect this. There must be evidence that prey animals are able to differentiate between threatening and non-threatening stimuli, and to change or mask their behaviour in relation to this.

This section will look at predator-prey interactions, and whether there is evidence to support the claims of the veterinary and ecology literature.

2.2.1 The selection of prey

It is largely assumed that predators are able to detect abnormal behaviour, or disadvantaged individuals. There are few empirical data to confirm that vertebrate predators select the most vulnerable rather than the most available prey, i.e. that they forage optimally. This scarcity of data is due largely to predators being difficult to study, and predation events difficult to witness, under natural conditions (Quinn & Cresswell, 2004).

The little information that is available is focused on the possible methods used by predators to select disadvantaged prey. Predators may select prey based on their own hunting methods, and the physical appearance or behaviours of prey animals.

2.2.1.1 Hunting methods

The method of predation adopted by a predator may act as a test for the fitness of prey. Canids such as wolves, are coursing predators, and typically pursue prey for long periods through relatively open terrain (Kruuk, 1972; Schaller, 1972). Because canids usually chase swift prey, capture success tends to be low, the individuals caught are generally disadvantaged in some way (Schaller, 1972), and kills are likely to occur when prey become exhausted or exhibit discernible disadvantages (Husseman et al., 2003). Whilst predators may be fooled by an injured animal running normally, it is the pressure of running for long lengths of time that reveals an animal's injury, or weakness.

Felids, in comparison, generally stalk prey and rely more upon cover to remain concealed prior to a chase, or a pounce. Therefore, the absence of a prolonged chase in felids should favour random choice of individuals from a prey population (Husseman et al., 2003), providing the prey effectively hide any afflictions.

Coursing predators have more opportunity to select animals in poorer condition than stalking predators due to their ability to select particular prey animals after the chase has been initiated. Wild dogs take a greater proportion of Thomson's gazelles in poor condition than cheetahs do (FitzGibbon & Fanshawe, 1989). Husseman et al. (2003) found that wolves and cougars both select disadvantaged prey, however, wolves exhibited a greater overall tendency to select juveniles and malnourished individuals among both deer and elk prey than did cougars. In addition, in winter, wolves predominantly kill fawns and older age classes of white tailed deer (Pimlott et al., 1969, cited in Curio, 1976). When random samples of animals shot by hunters were compared with kills of predators, it was found that predators selected more animals with lower body condition disproportionately from their proportion of the population. White-tailed deer killed by wolves were found to have significantly more abnormalities than a sample killed by hunters (Pimlott et al., 1969, cited in Curio, 1976). More recently, Pole, Gordon, and Gorman (2003) found that African wild dogs selected individuals in poorer condition than those randomly shot by humans, as measured from bone marrow fat levels.

While the notion that predators disproportionately select disadvantaged individuals from the prey population has received widespread empirical support (Curio, 1976; Murray, 2002), other studies have found there to be no difference in the prey caught by different predation methods (Rohner & Krebs, 1996;

Wirsing et al., 2002 cited in Husseman et al., 2003). Some variation in the quality of prey caught by predators may occur as a result of group hunting. Group hunting can aid in the capture of larger prey (Husseman et al., 2003). However, it is possible that the discrepancy in results is not solely due to the methods of hunting. Environmental factors can influence whether animals select compromised prey. These factors can include visibility, and the density of ground cover (FitzGibbon & Fanshawe, 1989).

2.2.1.2 Physical appearance

Wild animals that are sick may be more susceptible to predation, loss of social position, and displacement from territory holdings (Hart, 1988). Predators may use the appearance of a sick animal as an indication of physical condition. A coat that is dirty, oily and scruffy is an indication of a lack of grooming. Grooming has several functions including cleaning the fur of dirt and oils which improves its insulating efficiency (Thiessen, 1988, cited in Hart, 1988). By reducing the time they spend grooming, sick animals are able to conserve valuable energy, heat and water resources that can be used to combat fever (Hart, 1988), and thus they generally have a scruffy-looking coat. A dirty coat may not be the only sign of a reduction in grooming. A reduction in grooming can also lead to an increased parasite load, further accentuating the animal's poor condition. It is therefore possible that predators are able to determine the condition of their prey not only by a scruffy coat, but also by an increased external parasite burden, such as more ticks.

In the Serengeti National Park a higher proportion of male Thomson's gazelles were taken from the population than expected, which suggests that the poor physical condition of male Thomson's gazelles make them more susceptible to predation (FitzGibbon, 1990b). Males maintained lower fat reserves than females, especially during the rut, as a result of intraspecific competition and territory defence. In addition, Schaller (1972) noted that male Thomson's gazelles were more susceptible to sarcoptic mange and found a greater proportion of males dying or dead from disease. It is possible that predators detect this poor condition and increased level of disease, and concentrate on male gazelles.

Sickness *per se* may not directly enhance vulnerability, but it may stimulate prey to move into places that invite attack. Thus, whilst still swimming quite normally, injured fish seek the surface, and are exposed to more attacks from kingfishers than healthy specimens at greater depths (Kniprath, 1969, cited in Curio, 1976).

Toxoplasmosis infestation alters the behaviour of its intermediate hosts, mice and rats, such that they are more likely to be preyed upon by cats, enabling the life cycle of the parasite to be completed (Webster, 2001). Toxoplasmosis causes mice to be more active, to prefer exposed or novel areas, and to groom less, and it reduces their learning capacity (Webster, 2001). When given a choice, rats with toxoplasmosis, strongly prefer bedding that has been treated with cat urine over their normal bedding or bedding treated with rabbit urine (Webster, 2001). This is extremely abnormal behaviour for rats, as they are neophobic, reacting to novel stimuli with extreme caution and often total avoidance (Webster, 2001). Abnormal behaviour like this can make a diseased animal easy prey for predators.

Predators may also select animals displaying obvious injuries. Birds such as jays that occasionally hunt songbirds react quickly to a small bird wriggling in a net; seemingly oblivious to a human standing close by (Curio, 1976). The sight of distressed prey are so obvious and appealing to predators, that prey exhibiting any sign of impaired ability are detected quickly. In addition, certain species of birds use a 'broken wing' display when potential predators approach their nests. Adult ground-nesting birds such as killdeer and meadow pipits meander away from their nests trailing an 'injured' wing on the ground in the presence of a predator (Brunton, 1990). This draws the predator's attention toward it and away from the nest; diverting danger from the bird's offspring (Weary & Fraser, 1995). This display is directly mimicking a sick or lame animal, and the widespread occurrence of this mimicry suggests that many vertebrate predators can identify and will prey upon sick or disabled individuals if the opportunity arises (Curio, 1976).

2.2.1.3 Predators may select prey based on their small size.

Ungulates are most vulnerable during their first few weeks of life (Geist, 1971; Lent, 1974, cited in FitzGibbon, 1990a). Juveniles lack the speed and stamina of adults to outrun predators; and they are vulnerable to a greater range of predators as a result of their smaller size (FitzGibbon, 1990a).
Female Thomson's gazelles are rarely successful in actively defending their young, except sometimes from jackals (Wyman, 1967, cited in FitzGibbon, 1990a), and their fawns use hiding as their main means of defence (Walther, 1969, cited in FitzGibbon, 1990a). Even though they provide a smaller meal than adults, younger gazelles and wildebeests are still a lot easier to catch than mature animals, and they can be selected on the basis of their size before a chase has been initiated (FitzGibbon & Fanshawe, 1989). Thus they are more heavily preyed upon (Fanshawe & FitzGibbon, 1993), and form a large part of many predators' diets.

Temple (1987) suggests that there are two possible strategies that predators may implement when hunting prey, in order to select disadvantaged individuals: A predator could preferentially attack substandard individuals more frequently than normal individuals because substandard individuals may be conspicuous, and reveal their altered condition by some cue that the predator learns to associate with an easy capture, as outlined above. Alternatively, a predator may attack substandard and normal individuals in proportion to their occurrence in the population, but the success rate with substandard individuals could be higher. Which of the two strategies actually occurs is unknown; however, a mixture of both strategies could be possible. This concept needs further research.

2.2.1.4 *Behaviour changes*

The time an animal spends in vigilance behaviour may also be used by predators to assess prey condition. Vigilance is the term used to describe the upright searching posture that animals adopt to survey their environment for potential danger such as predators. Animals foraging alone tend to remain close to covered areas such as bush, so that they can escape quickly. When animals forage in groups, individuals take turns at being vigilant, allowing the majority of the group to feed. Group animals such as sheep become less vigilant as group size increases (Frid, 1997). The group as a whole benefits from this, as they are able to safely forage more efficiently, in riskier, open areas. A more vigilant individual might be safer, but excessive vigilance may also bring an unnecessary loss of feeding opportunities.

By selecting the least vigilant individuals in a group, predators are predicted to increase their hunting success for two reasons (FitzGibbon, 1989): First, such animals are likely to react more slowly to the predators' final attack. Second, since animals facing starvation are expected to maximize energy intake by spending more time feeding at the expense of other activities, in particular vigilance, less vigilant animals may be in worse condition, and therefore less able to escape the advances of a predator.

2.3 The Response Of Prey Animals To Threatening And Non-Threatening Stimuli.

In the previous section the ability of predators to select disadvantaged prey was discussed. In this section the ability of prey animals to distinguish between threatening and non-threatening stimuli will be discussed. If prey animals are able to distinguish between such stimuli, it is possible they are also able to alter their behaviour in response to these stimuli.

The literature based on the response of prey animals to a range of threatening stimuli is small. Studies in this area are generally concerned with the fear responses of prey animals (Bouissou & Vandenheede, 1995; Erhard, 2003). It has been shown that prey animals can differentiate between threatening and non-threatening stimuli. For example, Hansen et al. (2001) found that when shown a live dog, sheep took longer to behave normally again (to show no interest in the test apparatus anymore) in comparison to a human, a novel stimulus, and three stuffed predators (bear, wolverine and lynx). This is in accordance with Mortensen (1990, cited in Hansen *et al.*, 2001), who found that ewes were more attentive, showed a higher heart rate, and had a longer and faster flight reaction towards a wolverine model on a trolley than towards the trolley alone. Sheep that were presented with a dog, a human, a goat and a cardboard box exhibited the highest fearfulness scores in relation to the dog (Beausoleil et al., 2005). In addition, Tammar wallabies exhibited more foot thumps, which are considered the highest level of antipredator behaviour, when shown a model fox than to other stimuli (Blumstein, Daniel, Griffin, & Evans, 2000). The results of these studies indicate that prey animals are able to differentiate between stimuli, and that their reactions change depending on how much of a threat they view the stimulus to be.

2.3.1 Masking behaviour

As an evolutionary adaptation, animals may not exhibit behaviours associated with pain (Underwood, 2002), or injury. This behaviour may be called masking behaviour. Some animals may freeze in response to a predator, whilst others may flee, hide, or fight back. The survival of a prey animal thus depends on its ability to discover the predator, freeze, defend itself, or flee.

Some animals normally considered as prey species may show minimal outward signs of pain, and hide or remain motionless if attacked and injured. Others however, may attempt to escape, especially from where the pain was experienced, and travel rapidly for some distances in spite of severe injury (Short, 1998). Mortensen (1990, cited in Hansen et al., 2001) found when using a wolverine predator model that flight is the most common response among sheep, and that sheep in flocks respond earlier than solitary individuals.

Domestication and the degree of socialization an animal has experienced may influence the level of overt behaviour an animal will display in response to pain (Livingston, 1994, cited in Conzemius et al., 1997). Moreover, differences in behaviour may not be solely attributed to predator influence. If an individual is a high ranking animal in its social group, it may be in its best interest to hide pain or any sign of compromised ability from threatening conspecifics, in an attempt to maintain resources such as territories and mates.

Animals in pain behave in different ways depending on the site, severity and type of injury involved. However, animals also behave in a species-specific way (Flecknell, 2000). Some species may show obvious pain-related behaviour, such as loud vocalizations. For these animals it may be advantageous to encourage the help of conspecifics. Parents may help individuals who are attacked or in pain. In these circumstances distress signals would be adaptive when pain resulting from an injury is felt (Dobromylskji et al., 2000). For other species, pain is not expressed, even though it may be felt, because expressing such overt behaviour would simply alert predators that they were less fit and hence easy prey (Flecknell, 2000). As a result, these animals do not vocalize when injured (Broom, 2001). Therefore, in some cases signalling internal states may be maladaptive. For example primates might be particularly vulnerable to predation when giving birth; although there are many indications that this process is painful, it is rarely accompanied by vocalizations (Lefebvre & Giancarlo, 1985, cited in Weary & Fraser, 1995). In addition, a horse with a broken leg may graze normally (Bateson, 1991), or flee as if nothing is wrong.

Physiologically, animals may not exhibit clinical signs of pain initially, because of the secretion of naturally occurring analgesic opioid peptides. This is a natural mechanism enabling the severely injured animals to escape and not to have overt responses that would make them vulnerable to predation (Anil et al., 2002).

If overt pain-related behaviour is expressed, then the animal may mask this behaviour when it is aware of being observed. Animals may also change their responses in a familiar environment, and express less pain-related behaviour than when in an unfamiliar environment (Dobromylskyj et al., 2000). In a veterinary setting, this masking of pain behaviour makes assessment of an animal's condition problematic.

The literature is scarce on the masking of pain behaviour; however it is often assumed that animals do mask their behaviour in certain situations. From the little evidence there is, it is quite possible that animals can alter their behaviour according to different situations, including hiding behaviours that may make them more vulnerable to predators. But these presumptions need to be tested, and further research is required, as they have major implications concerning animal welfare and the way animal pain is currently regarded. If animals do not exhibit pain-related behaviour following a painful experience in the presence of a predator, then it may be difficult to use behaviour to assess whether certain procedures are painful, and whether analgesics are working effectively. Because observing overt behaviour is the most commonly used method of assessing animal pain, the possibility that animals can mask pain results in problems in the use of this method of assessment of pain.

2.4 Responses to Predators and Stimuli

2.4.1 Sheep and their predators

The anti-predator behaviours of sheep can be broadly divided into those responses that are elicited by the presence of a predator, and those that have evolved to reduce the chances of detection and capture (Dwyer, 2004). In some cases, the same behaviour may be involved in both responses.

Predators of wild and domestic sheep include lynx, mountain lions, coyotes, dingoes, wolves, wolverines, foxes, brown bears, eagles and other predatory birds (Hansen et al., 2001; Dwyer, 2004). In addition, domestic dogs are widely documented as predators of sheep (Boggess, Andrews, & Bishop, 1978; Robel, Dayton, Henderson, Meduna, & Spaeth, 1981). Predators preferentially kill lambs, juveniles, females, and individuals with reduced locomotor capabilities in wild and domestic sheep populations (Gluesing, Balph, & Knowlton, 1980). Coyotes prey disproportionately on lambs that behave abnormally (e.g. limping, hunched, lethargic), on lambs from ewes that are not healthy, and on single rather than twin lambs (Gluesing et al., 1980). Healthy lambs that are killed generally display more investigatory and playful behaviours than their surviving counterparts (Gluesing et al., 1980). In general, predators appear to prey most heavily on animals that are on the periphery of the social group (Dwyer, 2004).

From these findings, there appear to be at least two important underlying factors influencing the survival of prey: social status and impaired defence capability. Gluesing et al. (1980) noted that disabled lambs and disabled or diseased ewes lagged behind the flock and were observed on the periphery of the bed-ground. It is possible that these animals are on the periphery of the group because their social status has decreased as a consequence of their poor health. This is supported by the findings of Lynch & Alexander (1973, cited in Dwyer & Bornett, 2004), who found that subordinate sheep were commonly at the back of the moving group, and that these animals were likely to eat poorer quality feed, leading to higher parasite burdens. In addition, lambs with no abnormalities from disabled ewes may have been killed disproportionately more than other lambs because they were likely to be on the periphery of the herd, and also because their mothers were less able to defend them due to their poor health. Thus, the social group and perhaps social status is important in minimizing individual predation risk, and it seems likely that there will be strong selection pressure on prey species to appear behaviourally similar to the other members of the group since abnormal animals are preferential prey.

2.4.2 Sheep behaviour in response to predation

Sheep are a highly vigilant species and will normally flee when a threat is perceived. Individuals are strongly linked to the flock, and the initial movement of a few sheep may result in the whole flock moving away (Lynch et al., 1992, cited in Wemelsfelder & Farish, 2004). Sheep may use specific signals; perhaps as ‘pursuit deterrents’ to inform the predator that it has been detected.

These include snorts, orienting towards the disturbance, stotting, and stiff-legged bouncing (Geist, 1971; Schaller, 1977).

Upon detecting a potential predator or other disturbance, sheep typically show an 'alarm-posture' (Geist, 1971). This is a statue-like posture in which the animal stares at and orientates its ears in the direction of the disturbance. The sheep may not immediately run away, but may walk in a stiff gait, with its head held up rigidly. It may stop periodically, look at the source of disturbance and strut on again. It may also stamp the ground with its front leg and blow sharply through its nose. This type of behaviour has the effect of drawing the attention of other sheep, which will then cease their behaviour, and also express alarm (Geist, 1971). If the threat persists, the animals will often come together as a flock, and run away. The formation of a group in the presence of a predator may occur as each animal attempts to move towards the central position in the flock, as animals on the outside of the group are at a greater risk (Gluesing et al., 1980); resulting in a circling aggregation (Vine, 1971, cited in Dwyer, 2004). This circling movement reduces the predation risk of animals in the centre of the herd if the predator is likely to kill only the animal first encountered in the group (Triesman, 1975), and it may reduce predator capture efficiency through a confusion effect (Dwyer, 2004).

Wild sheep show strong antipredatory behaviour (Geist, 1971). They are pronounced flock animals, shy, attentive and with quick flight reactions, while defending their offspring when necessary (Berge, 1942, cited in Hansen et al., 2001). In comparison to wild species, domesticated species show a reduced alertness and attentiveness to the environment and reduced flight distances (Price, 1984; Hemmer, 1990), but increased social behaviours (Kunzl & Sachser, 1999, cited in Dwyer, 2004). However, both mountain and domestic sheep will defend their young from the advances of small predators such as crows and foxes by standing over their lamb (Geist, 1971).

Wild and domestic sheep differ in the amount they vocalise. The survival costs of using vocal communication mean that wild sheep rarely use vocalisation as a signal. Vocalisations in wild sheep appear to be inhibited in fear situations

(Kiley, 1972, cited in Dwyer, 2004), in which sheep instead rely on visual forms of communication. The vocal behaviour of domestic sheep in comparison has increased in frequency with the degree of domestication (Kiley, 1972, cited in Dwyer, 2004). It is possible that the frequency of vocalisation has increased because domestic sheep are farmed in larger groups, and this may require more complex social signals (Berger, 1979). Also, because the risk of predation is reduced in domestic sheep, there is no longer selection pressure against vocalisation (Price, 1984). Social isolation and feeding times tend to invoke vocalisations in more intensively managed breeds of domestic sheep (Shillito-Walser, Walters, & Hague, 1982), but vocalizations tend to be inhibited in other situations, for example in the presence of a tethered dog (Torres-Hernandez & Hohenboken, 1979). This parallels the behaviour of wild sheep in the presence of a predator (Kiley, 1972, cited in Dwyer, 2004).

2.4.3 Responses of sheep to fear-eliciting situations

The tendency of animals to react to potentially threatening events, specifically by fearfulness, is an aspect of emotional reactivity and temperament. Fear reactions are obviously adaptative in the wild; however, intense or chronic fear reactions in breeding animals can lead to reduced productivity and poorer welfare (Vierin & Bouissou, 2002).

A series of fear-eliciting standardised tests have been developed, and used to quantify a number of behaviours interpreted in terms of fear for sheep (Romeyer & Bouissou, 1992). These tests use a combination of stimuli classically reported to induce fear: isolation from conspecifics, a surprise effect, novelty, and the presence of a human. When sheep were confronted with novel or unexpected events under controlled experimental conditions, their response was characterised by immobilisation, glancing at the stimulus, shivering and foot-stamping, defecation, high-pitched bleats, low levels of feeding, high levels of locomotion, and repeated attempts to escape the enclosure (Romeyer & Bouissou, 1992; Vandenheede, Bouissou, & Picard, 1998).

During routine husbandry, such as shearing, gathering, and handling, sheep are usually moved using fear-evoking stimuli (Gonyou, 2000; Hutson, 2000). Many sheep, particularly those kept under extensive conditions are worked with dogs, usually for herding rather than for guarding (Coppinger & Coppinger, 2000), often with other frightening stimuli, to elicit a flight response. The presence of a dog or recorded dog barking is used as a stressor in experimental studies and causes elevations in plasma cortisol, ACTH, heart rate and body temperature above those observed upon exposure to humans and noise (Harlow et al., 1987; Baldock & Sibley, 1990; Cook, 1996; Komesaroff et al., 1998, cited in Dwyer & Bornett, 2004). Beausoleil et al. (2005) found that sheep were highly vigilant, exhibited little exploratory behaviour and foot-stamped when a dog was present in an arena test (Beausoleil et al., 2005). In contrast, sheep explored the arena frequently in the presence of a human, a cardboard box, or a goat. In the presence of the box, this exploration was also extended to the stimulus. Furthermore, in the presence of the goat, sheep were highly vocal, and often sniffed the goat. These findings demonstrate that sheep regard dogs as predators, and are able to distinguish between threatening and non-threatening stimuli.

Several factors affect how an animal perceives a stressor and the type of response initiated by an animal. On the basis of factors identified by Moberg (1985), examples from the literature of how the physiological and behavioural responses of sheep to stressors vary with these factors are given below:

2.4.3.1 Experience

Sheep quickly learn to associate unpleasant experiences with places, people or auditory stimuli (Fell & Shutt, 1989; Rushen, 1996; Mears et al., 1999, cited in Dwyer, 2004), and they show long-term avoidance of these stimuli (Hutson, 1985, cited in Dwyer, 2004). This behaviour is not exhibited by naïve animals. The ability to rapidly learn about unpleasant stimuli is highly adaptive for a prey animal, and might aid survival through avoidance of places where predators have been encountered (Dwyer, 2004). Schaller (1977) suggested that the experiences of wild sheep may alter their responsiveness to predators. By watching the behaviour of predators, prey animals may be able to assess the likelihood of attack. Obviously, it is adaptive for prey animals to respond only to definite threats, rather than to expend valuable energy and forego foraging opportunities in unnecessary flight (Dwyer, 2004). In domestic sheep, adult ewes responded with aversion to images of dogs, whereas young lambs did not (Porter & Boussiou, 1999), suggesting that the lambs had not yet had enough experience with dogs to link unpleasant associations to these images (Dwyer, 2004).

Sheep are able to recognise the faces of sheep of the same breed, familiar animals, dogs and humans (Kendrick & Baldwin, 1987; Kendrick, 1991). Although the faces of humans and dogs are not physically similar, they appear to represent a similar threat to sheep (Kendrick & Baldwin, 1987). However, Peirce, Leigh, DaCosta, and Kendrick (2001) showed that the sheep's brain responds to socially familiar sheep as it responds to images of familiar stockpersons, but not to images of other humans. Sheep can distinguish between different handlers on the basis of their previous experience (Fell & Shutt, 1989; Boivin et al., 1997; Davis et al., 1998, cited in Dwyer, 2004). Furthermore, guard dogs have been effectively used to guard sheep flocks (Andelt & Hopper, 2000, cited in Dwyer, 2004), suggesting that sheep may view some dogs differently depending on their experience of them (Dwyer, 2004). Thus, the way sheep view potential predators appears not to be innate, but is instead dependent on the previous experience of the sheep (Dwyer, 2004), indicating that sheep have long-lasting memories for both negative and positive experiences (Rushen, 1990; Grandin, 1997).

2.4.3.2 Genotype

Sheep of different breeds tend to react to stressors in different ways, some more severely than others and there are many individual variations in the stress response within breeds (Cockram, 2004). For example, less highly selected breeds are more fearful than are highly selected breeds – they make more lowpitched but less high-pitched bleats in the test arena, feed less often in the presence of an observer, are more immobile, and urinate or defecate more frequently (Romeyer & Bouissou, 1992; Le Neindre et al., 1993; Lankin, 1997, cited in Dwyer, 2004). In the presence of stuffed predators, more primitive lightweight breeds of sheep have greater flight distances, tighter flocking behaviour and a longer recovery time than more highly selected heavier breeds (Hansen et al., 2001). The less selected hill and upland breeds or more primitive breeds appear to have a greater reactivity to the same stressor and take longer to recover than more highly selected heavier breeds (Dwyer, 2004). To further illustrate this point, Vierin & Bouissou (2003) found that Romanov ("light" breed) sheep were more fearful than Ile-de-France ("heavy" breed) sheep when subjected to the fear-eliciting tests developed by Romeyer & Bouissou (1992).

2.4.3.3 Sex and Age

The responses of sheep to stressful stimuli may differ as a consequence of their age or their sex. For instance, Connolly et al. (1976, cited in Cockram, 2004) found that rams behaved differently in response to coyote predation than ewes. This has been supported by other studies, which have found ewes to be more fearful than rams (Vandenheede & Bouissou, 1993; Vierin & Bouissou, 2003). In addition, ewes and juveniles are more likely to flee from a predator threat than are male sheep (Bleich, 1999, cited in Dwyer, 2004), and ewes are more vigilant than males (Schaller, 1977; Laundre et al., 2001, cited in Dwyer, 2004). Since predators preferentially select females and juveniles, these behavioural responses suggest that the sheep are responding to the relative risk of predation (Dwyer, 2004).

Juvenile Bighorn sheep (*Ovis canadensis*) are more likely to flee in the presence of predators than are older sheep (Bleich, 1999, cited in Dwyer, 2004). This suggests that young sheep are probably more fearful than older animals of stimuli associated with predation (e.g. the presence of human observers). Experimental data from domestic sheep support this, since older ewes have lower ‘panic’ reactions to humans than do younger ewes (Vandenheede et al., 1998), are less likely to flee when their lambs are handled by an observer (Lambe et al., 2001, cited in Dwyer, 2004), and are less reactive to humans in tests. Furthermore, three- to four-month old lambs are more fearful than five- to six-month old lambs (Vierin & Bouissou, 2003). These results contrast with the findings of Porter & Boussiou (1999), as mentioned earlier, who suggested that lambs may not have had enough experience with dogs to link unpleasant associations with images of dogs.

2.4.3.4 Physiological State

Physiological changes during the reproductive cycle such as pregnancy, parturition and lactation can result in altered reactions to stress in sheep (Cockram, 2004). Sex hormones may also alter the fear reactions of animals. Pregnant Ile-de-France ewes exhibited reduced fearfulness in comparison to controls, and it is thought that this decreased fearfulness has adaptive value for the survival of their young (Vierin & Bouissou, 2001). During pregnancy, ewes are more likely to forage in areas containing better grazing to meet their increased nutritional demands (Dwyer, 2004); even if the risks of predation are greater in these areas (Berger, 1991). At the onset of birth however, pregnant ewes retreat to secluded parts of their home range where predation risks are minimised (Geist, 1971; Schaller, 1977). Some days after the birth, ewes and their lambs rejoin the flock but remain in close proximity to escape terrain (Berger, 1991), and remain more vigilant than non-lactating ewes (Dwyer, 2004).

Ewes with lambs may display aggressive behaviours towards shepherds and dogs, and will maintain a close proximity to their lambs at the approach of a human (O’Connor, Jay, Nicol, & Beatson, 1985). These responses may result from either a reduction in fearfulness or an increase in aggression (Lawrence et al., 1998, cited in Dwyer, 2004), and provides a striking contrast to the behaviour of non-lactating sheep to stressful stimuli (Dwyer, 2004). Maternal experience also influences the fearfulness of sheep. Vierin & Bouissou (2002) compared the fear reactions of three types of sheep – those that had not raised a lamb, those that had raised one lamb and those that had raised one or more lambs. Having one lamb significantly reduced the fear reactions of ewes, but it was the combination of several maternal experiences and age that appeared necessary to reduce the fear of humans (Vierin & Bouissou, 2002).

In addition, sex hormones may alter the fearfulness of sheep, following the findings that wethers are more fearful than rams (Vandenheede & Bouissou, 1996), and that testosterone treatment reduces fear reactions of ewes (Vandenheede & Bouissou, 1993).

2.5 Responses to Painful Stimuli

There are three types of scientific protocol that can be used to determine how an animal responds behaviourally to different painful stimuli, which behaviours indicate pain, and to evaluate the severity and alleviation of pain. These are: (1) Observational studies where animals are subjected to specific painful treatments, or injured or diseased animals are compared with control groups and groups receiving effective analgesia; (2) Trials which measure the response of treated and control animals to defined painful pressure, thermal or electrical stimuli; and (3) Preference studies where animals are allowed to choose painful or non-painful treatments or to self-medicate with an analgesic to alleviate pain.

Two of these three protocols will be reviewed here. Observational studies on lambs after castration and tail docking, and the change in thresholds to mechanical pressure stimuli will be reviewed as examples of (1) and (2) respectively.

2.5.1 Sheep and their response to castration and tail docking

Lamb castration and tail docking are common husbandry procedures, and are usually performed on lambs at three to six weeks of age. Ram lambs are castrated to avoid aggressive behavioural problems and indiscriminate breeding (Lester, Mellor, Ward, & Holmes, 1991), whilst tail docking is performed to decrease the prevalence of fly strike (Lester et al., 1991) by keeping the tail area free of faeces. There are many methods that can be used to castrate and tail dock lambs, including rubber rings, burdizzo clamp or bloodless castrator, searing and cutting using a heated docking iron (tail docking only), or surgical removal. The rubber ring method is one of the most common methods of castration and tail docking (Thornton & Waterman-Pearson, 2002), in both the UK (Archer, Johnston, & Khalid, 2004) and NZ. This technique is popular with farmers as it is quick, easy, economical and effective (Archer et al., 2004).

Castration and tail docking, which are known to cause distress and pain, are performed without anaesthesia or analgesia (Pollard, Roos, & Littlejohn, 2001). This has raised concerns regarding the welfare of the lambs involved. In response to this issue, over the last fifteen years, the pain-induced distress caused by castration and tail docking methods has been investigated extensively in lambs (Lester et al., 1991; Dinniss, Mellor, Stafford, Bruce, & Ward, 1997; Thornton & Waterman-Pearson, 1999; Molony et al., 2002). Various measures have been used to assess the level of pain experienced by lambs following castration and tail docking. These include physiological and behavioural measures.

2.5.1.1 Physiological measures

A rise in plasma cortisol concentration is a commonly used physiological indicator of pain. Measurement of plasma concentration of cortisol have shown that castration produces increases in cortisol concentrations significantly greater than in control lambs, and that surgical castration produces a greater peak and duration of cortisol increase than rubber ring castration (Lester et al., 1991; Kent et al., 1993). Unfortunately, to measure physiological indices such as plasma cortisol concentration, the collection of blood samples is necessary, and involves handling the animals. Handling alone has been found to induce stress and to lead to an artificial rise in the level of plasma cortisol (Haresign, Williams, Khalid, & Rodway, 1995; Khalid, Haresign, & Bradley, 1998; Jongman, Morris, Barnett, & Hemsworth, 2000). In comparison, the assessment of pain through the monitoring of behaviour avoids additional variables resulting from handling. The selection of appropriate parameters is also relatively straightforward as a result of the extensive studies and classifications of both normal and abnormal behaviour in sheep (Mellor & Murray, 1989). Furthermore, at least one study has found active pain behaviours to be more successful in discriminating between the severity of painful treatments than the measurement of plasma cortisols (Molony & Kent, 1997).

However, it is necessary to use physiological indices of distress intensity, such as cortisol responses, in order to interpret behavioural observations because no single parameter of either behaviour or physiology is sufficient to make an objective assessment of the level of pain being experienced by the animal. In addition, when the two measures are used together, the results can be validated against each other.

2.5.1.2 *Behavioural measures*

Behavioural and postural indicators have been used to define the pain response in lambs following husbandry procedures (Mellor & Murray, 1989; Molony & Kent, 1997; Thornton & Waterman-Pearson, 1999; Molony et al., 2002). Behaviours commonly associated with castration and tail docking in lambs include licking or biting the site of damage, lip-curling, and increased activity such as pacing, rolling, writhing, jumping, kicking, stamping and tail-wagging (Molony & Kent, 1997; Dinniss et al., 1999; Thornton & Waterman-Pearson, 1999). There is an increased frequency of abnormal behaviours and abnormal postures in castrated lambs compared to control lambs. Behaviours such as those listed above are otherwise rarely exhibited by lambs, and are therefore regarded as signs of discomfort and distress associated with pain (Dinniss et al., 1999).

Behaviour that reflects attempts to escape or eliminate a perceived threat may be regarded as a specific indicator of pain, and furthermore, the level of behavioural agitation associated with these actions may reflect the severity of the experience (Molony & Kent, 1997). Therefore, the more intense the pain, the more vigorous the attempts to escape or eliminate its source should become (Molony & Kent, 1997). Lambs soon become tired of trying to escape the pain, and this leads them to alter their strategy to more passive ways of avoidance (Molony & Kent, 1997). Following castration and tail docking, lambs adopt abnormal postures and walks, including standing with a hunched back, lying with legs fully extended, and walking with an unsteady gait. They increasingly refrain from active movement, and sometimes stand or lie completely still (Molony & Kent, 1997). Lambs exhibiting these behaviours may lack alertness and fail to exhibit normal flight behaviour; they appear drowsy and apathetic and do not respond to vigorous nudging by the ewe (Molony et al., 1993; Dinniss et al., 1999; Thornton & Waterman-Pearson, 1999). Molony et al. (1993) suggest that standing or lying still may reduce the intensity of pain because a lack of movement minimises stimulation of damaged and sensitised tissues. So in this case, the more intense the pain, the more still and inert the animal would become, while switching off its normal levels of sensory responsiveness.

The behavioural expression of pain in sheep can be separated into two distinct components: an expression of struggle and nervous agitation, directed at the perceived source of pain, and, at a later stage, the adoption of a more passive strategy as the animal appears to give up the fight. In this area of research, the qualitative assessment of pain behaviour plays an important role and has been successfully applied as a measurement tool.

Thornton and Waterman-Pearson (1999) developed a comprehensive visual analogue scale (VAS) to study the different types of pain related behaviour of lambs following castration. This scale used a more qualitative approach, assessing three types of behaviour: active pain behaviours such as lip-curling; the response of lambs to interactions with the observer; and the animal's response to palpation of the scrotal area. The results from this scale gave more detailed information about the behaviour of lambs than observations of active pain behaviours alone, which had been favoured by previous researchers (Molony et al., 1993; Lester et al., 1996; Dinniss et al., 1999). In addition, these ratings effectively distinguished between the animal's response to different castration methods (applied with or without anaesthetics), which suggests that they were reliable and sensitive indicators. A similar result was achieved using qualitative rating scales of post-operative pain in cats (Cambridge, Tobias, Newberry, & Darkar, 2000). It has been suggested that qualitative rating scales may be able to overcome many of the limitations inherent in conventional quantitative approaches (Thornton & Waterman-Pearson, 1999).

In order to evaluate alternative husbandry procedures or implement pain minimisation strategies, the welfare impact of these procedures needs to be assessed objectively. Unfortunately, obtaining objective measures of pain or pain relief is difficult. Active pain behaviours have been used to investigate the painfulness of castration and tail docking by various methods, including burdizzo and tight rubber rings (Molony et al., 1993; Kent, Molony, & Robertson, 1995; Molony et al., 1997). They have also been used to examine the influence of local anaesthetic on reducing these painful behaviours following castration and tail docking (Wood et al., 1991; Graham, Kent, & Molony, 1997; Kent, Molony, & Graham, 1998). However, different procedures generate different pain types, and thus different quantities and qualities of pain. The extent of the pain response of most treatments appears to be based upon the amount of tissue involved and the degree of enervation at the site, whereby tail docking<castration<tail docking and castration combined (Lester et al., 1996; Molony & Kent, 1997; Molony et al., 2002).

Different husbandry methods can also produce different expressions of the pain response. The use of rubber rings blocks blood flow, causing ischaemia and the death of tissue distal to the ring (Rutherford, 2002). This results in an increase in abnormal behaviours and postures. Surgical husbandry treatments cause the greatest rise in physiological indices of pain (Mellor & Stafford, 2000), and result in increased abnormal postures and a reduction in activity (Shutt, Fell, Connell, & Bell, 1988; Molony et al., 1993; Lester et al., 1996). The abnormal behaviours that follow castration or tail docking indicate that acute distress lasts for three and a half hours or less in ring-treated lambs, and about eight hours in surgically treated lambs (Lester et al., 1996; Dinniss et al., 1997). These results therefore suggest that the use of rubber rings to castrate or tail dock lambs causes less overall acute distress than surgical castration (Lester et al., 1996).

The use of local anaesthetic in conjunction with methods such as rubber ring castration and bloodless castration has been shown to abolish acute behavioural and cortisol responses (Wood et al., 1991; Graham et al., 1997; Molony et al., 1997; Kent et al., 1998; Sutherland et al., 1999; Thornton & Waterman-Pearson, 1999). However, local anaesthetic was not successful in eliminating the reactions to surgical castration, but these reactions were effectively eliminated by general anaesthetic.

It has been suggested that the use of a rubber ring and a distally placed bloodless castrator (which crushes sensory afferents) is one of the most effective methods of reducing acute pain following castration (Molony et al., 1997), and tail docking (Graham et al., 1997). This method reduces the abnormal behaviours and postures as well as the peak and duration of the cortisol rise seen in lambs castrated by rubber ring alone (Thornton & Waterman-Pearson, 1999). Conversely, the use of a local anaesthetic, either injected or needleless, was found to be more effective in reducing pain than the application of the burdizzo or the clamp after application of the rubber ring, following the findings that bloodless castrators do not eliminate pain generation whilst the actual procedure is being performed (Sutherland et al., 2000), and that they are not as effective in reducing the pain of lambs over one week of age (Mellor & Stafford, 2000). Furthermore, some farmers are unwilling to use bloodless castrators due to the pain of application, and the flinching reaction of the lambs each time the clamp is applied (Molony et al., 1997; Mellor & Stafford, 2000).

Between breed differences in the response of sheep to pain have also been studied. Archer et al. (2004) found that Charolais cross lambs were more active, had higher respiration rates and took longer to recover than Suffolk cross lambs following castration and rubber-ring tail docking. These findings suggest that different breeds of lambs experience different levels of distress in response to the same husbandry procedures. Alternatively, the results may simply reflect a difference in the character and temperament of the breeds studied. Further studies are needed before any conclusions can be made, however if breeds do differ in their response to pain, categories of normal and abnormal behaviour would need to be allocated to each breed in order to improve the accuracy of any future assessments (Archer et al., 2004). From what is known of the differences of certain breeds to fear-eliciting situations based on the level of domestication of the breed (Hansen et al., 2001), it is likely that breeds may differ in their response to painful stimuli as well.

2.5.2 Sheep and their response to mechanical stimuli

Mechanical and thermal stimuli have been used to study acute and chronic pain in animals (Ley, Livingston, & Waterman, 1989; Welsh & Nolan, 1995;

Lascelles, Cripps, Mirchandani, & Waterman, 1995; Thornton & Waterman-Pearson, 1999). An example of a mechanical stimulus is a pneumatic pressure device that gradually pushes a blunt pinhead against the skin of the animal's leg. The threshold response to this stimulus is a clearly defined lifting of the leg. In comparison, an example of a thermal stimulus is a heating unit and a thermocouple positioned on the animal's body. If the thermocouple is positioned on the animal's ear, using an ear-clip, for example, the device delivers heat into the ear, and an ear-flick or shaking of the head defines the response point.

Studies have found that an animal's threshold to these mechanical or thermal stimuli change in response to chronic pain. This change indicates an alteration in nerve function or in nociceptive processing at a higher level (Nolan, 2000). Increases and decreases in noxious mechanical or thermal thresholds are interpreted as decreases and increases in sensitivity to mechanical or thermal stimulation respectively. By assessing the changes in nociceptive thresholds, the presence of hypoalgesia or hyperalgesia following procedures such as castration can be identified.

Thornton and Waterman-Pearson (1999) found that rubber ring castration of lambs did not significantly alter the threshold to a mechanical stimulus, however surgical castration, or castration by a combination of rubber ring and clamp produced reduced nociceptive thresholds for seven and four hours after castration respectively. Sheep with severe lameness have a significantly reduced threshold to mechanical nociceptive stimuli than healthy controls. However, there was no difference in the mechanical thresholds of less severely affected sheep and healthy controls (Ley, Waterman, & Livingston, 1995). The hyperalgesia of severely affected sheep was found to persist for at least three months, even though the sheep did not appear to be lame (Ley et al., 1995).

The thresholds of lame dairy heifers to a mechanical stimulus tested on the leg decreased during the post-partum period, but the response to a thermal stimulus applied to the ear was unaffected (Whay, Waterman, & Webster, 1997). Therefore, lameness causes hyperalgesia to mechanical stimuli in sheep and cattle. Interestingly, hyperalgesia to a thermal stimulus is induced following abdominal surgery in sheep but not to a mechanical stimulus when the stimuli were applied to the ear or leg respectively (Welsh & Nolan, 1995).

The measurement of the nervous response to a painful stimulus has been widely used as a means of testing the analgesic efficacy of drugs in animals (Ley et al., 1995). The thresholds to noxious mechanical stimuli in sheep with footrot were lower than in control animals, but they returned towards normal values after a local anaesthetic block (Ley et al., 1989), suggesting that a peripheral noxious input plays a role in the hyperalgesia detected.

2.6 Conclusions

In this review, it has been shown that animals do feel pain, and that pain can be inferred from measurements of behaviour, and physiological data. Observing behaviour is the most commonly used method of assessing pain. However, physiological indices can increase the value of behavioural observations as an alternative means of validation, and as a means of adding objectivity.

Various approaches have been implemented to study and assess animal behaviour in relation to pain. These include the use of pain scales, motivation tests, observations of behaviour following treatment, individual and species-specific responses to pain and using analgesics and anaesthesia.

Whilst pain scales have been effective in the evaluation of human pain, they have proven to be unreliable in animals. The basis of the concept is good; however, more research is required to make a multidimensional pain scale that removes inter-observer variability. Motivation tests have also been used to evaluate pain in animals. The extent to which an animal responds to noxious stimuli may also be an indication by inference, of the pain experienced. Animals have been trained to avoid painful stimuli such as electric shocks, and other studies have shown that animals can choose to self-administer analgesics when they develop chronic painful conditions. In some studies of pain, operant methods have been used to test the motivation of an animal to escape or avoid a noxious stimulus.

In addition, it is possible that behaviour may indicate noxious sensory input and, by inference, pain, if it is exhibited by treated animals during or after treatment, but not by control animals. (Dinniss et al., 1999).

Behaviour associated with pain often appears very specific to the species involved, and to different types of pain (Lester et al., 1996; Dinniss et al., 1999). It is important in the diagnosis of pain in animals to appreciate the species, breed and individual differences in their response to injury (Short, 1998).

Analgesics and anaesthetics can also be useful in the assessment of pain. It is possible to use behaviour to identify nociception and by inference, pain, if effective local anaesthesia or analgesics return behaviour levels close to those exhibited by control animals (Dinniss et al., 1999).

Evidence has been shown that suggests predators do attack disadvantaged prey as stated by the veterinary and ecology literature. How predators select their prey can be a result of hunting methods used by the predator, or the physical appearance or behavioural changes exhibited by prey.

Whether a predator preferentially attacks substandard individuals more frequently than normal individuals because their altered condition can be easily detected by the predator, or whether a predator may attack substandard and normal individuals in proportion to their occurrence in the population, but have a higher success rate with substandard individuals is not known.

Prey animals are able to differentiate between stimuli, and these responses do change in relation to the level of threat the stimuli pose to the animal. However, there is no valid support for the claims of the veterinary and ecology literature surrounding the masking of pain behaviour by prey animals. There is a gap in the literature as to whether animals will mask pain in relation to the presence of a predator. From the little evidence available, it is quite possible that some animals can mask their pain or injuries in the presence of a predator. This masking behaviour can include the suppression of vocalizations in relation to a painful occurrence, and it may determine whether an animal decides to flee, freeze or defend itself in any given circumstance. This area needs to be further researched before the blanket claim of suppression of pain behaviour can be validated.

Sheep have a large number of natural predators, and they maintain a specific set of behaviours to use in relation to the presence of a predator, no matter whether they are wild or domesticated. These behaviours include the formation of groups, foot stamping, snorting, orienting towards the stimulus and fleeing.

Sheep view dogs (and related species such as wolves) as predators, and sheep can distinguish between threatening and non-threatening stimuli even after being domesticated for so long. The previous experiences of sheep also influence the way they view certain people and animals in relation to the level of threat they pose. In addition, how sheep react to different stimuli can be influenced by a number of factors including genetics, sex, age and physiological state.

A large number of studies have categorised a specific set of behaviours exhibited by lambs following castration and tail docking. These behaviours have been used to quantify the level of pain experienced by the lambs following these procedures, and have been found to be a reliable method of pain assessment.

Mechanical pressure thresholds have been used to assess the presence of hypoalgesia or hyperalgesia following painful experiences such as lameness and castration. Studies have found that animals remain sensitive to these pressure devices long after the physical symptoms have disappeared; suggesting that pain persists and can be detected, long after it appears to have subsided.

While the response of sheep to different stimuli is known, how these behaviours change when a sheep or lamb is in pain and a predator is present has not been tested. As outlined above, lambs will behave in an overt and active manner following castration and tail docking, and this will eventually subside to withdrawn, statue-like behaviours in an attempt to reduce pain. When lambs are in pain in the presence of a predator, the display of pain behaviours may conflict with the animal's desire to behave in a way similar to herd mates, and thus avoid predator detection (Broom, 2001).

Overt behaviour therefore may not be the best index of pain quantification because of these conflicting factors. If animals do not exhibit pain related behaviour, following a painful experience, in the presence of a predator then it may be difficult to use behaviour to assess whether certain procedures are painful and whether analgesics are working. Behaviour is the most commonly used method of assessing animal pain, and the possibility that animals can alter the amount of pain behaviour they display may cause problems with the use of this method of assessment, and with it, our understanding of pain.

2.7 Hypotheses and Aims

The aim of this thesis was to test the hypothesis that:
"The presence of a predator influences the behaviour of an animal in pain".

The objectives of this experiment were to determine whether the behaviours of recently castrated lambs differed more in the presence of a predator than other stimuli, and whether the presence of the lambs' dams had any further influence on these behaviours. In a second trial adult sheep were subjected to a mechanical pain stimulus to test whether their pain thresholds were altered in the presence of a predator.

The hypotheses to be tested were (1) That castrated lambs would behave differently from control lambs, and that castrated lambs would reduce their pain related behaviours more when a predator stimulus was present (dog or dog barking tape) than when a non-threatening or biologically insignificant stimulus (goat or cardboard box) was present. (2) That the presence of the lambs' dams would influence their pain related behaviours in the presence of the predator stimuli (dog or barking tape). (3) That adult sheep would increase their pain thresholds in the presence of predator (dog or barking tape).

3 The Effect of Predator Presence on Lamb Behaviour Following Castration

3.1 Introduction

The behaviour of individual sheep in response to the presence of dogs, humans and other stimuli and in various situations has been studied in terms of fear and aversiveness (Romeyer & Bouissou, 1992; Vandenheede & Bouissou, 1993, 1996; Bouissou & Vandenheede, 1995; Vandenheede et al., 1998; Beausoleil et al., 2005). However, these studies have not examined whether the presence of dogs or humans modify the behaviour of sheep when they are in pain. It has been suggested (Broom, 2001), that the pain caused by injury may motivate an animal to behave in a pain-reducing manner but that may conflict with the animals desire to behave in a way similar to conspecifics and thus avoid predator attention.

Lamb behaviour following castration has been studied (Mellor & Murray, 1989; Lester et al., 1996; Dinniss et al., 1999; Thornton & Waterman-Pearson, 1999; Molony et al., 2002), and a number of behaviours and postures are known to indicate pain in lambs. Castration using rubber rings is known to elicit characteristic behaviours, which are otherwise rarely seen in lambs (Mellor & Murray, 1989; Dinniss et al., 1999). These behaviours include; increased restlessness, lateral recumbency, writhing and kicking (Mellor & Murray, 1989; Mellor et al., 1991; Molony et al., 1993; Kent et al., 1995; Lester et al., 1996).

If Broom's (2001) hypothesis is correct, lambs should show fewer behaviours indicative of the pain caused by castration in the presence of aversive stimuli, such as a predator.

The objectives of this experiment were to determine if the behaviour of recently castrated lambs, would be modified by the presence of specific stimuli (dog, goat, cardboard box, and a tape recorder playing the sound of a dog barking), and whether the behaviour of recently castrated lambs would differ from those of control lambs in the presence of a predator, and finally, if lambs would alter their behaviour differently in the presence or absence of their dam.

The hypothesis to be tested was that the behaviour of castrated lambs would differ to that of control lambs, and that castrated lambs would show less castration pain behaviour in the presence of a predator (dog and dog barking tape), than when a biologically insignificant novel object (cardboard box) or a goat were present. Finally it was hypothesized that lambs would behave differently in the presence of their dam, when a predator was present because the dam would be more experienced, and hence cue her lamb in some way to the potential danger.

3.2 Methods and Materials

3.2.1 Introduction

Three hundred and eighty four lambs were used in this four day study. One hundred and ninety two lambs were studied without their dams, in groups of eight, on the first two days of the study (Table 3.1). Another one hundred and ninety two lambs were studied along with their dams, in groups of eight lambs and their eight dams, on the remaining two days of the study (Table 3.2). Over the four-day study period each group was exposed for thirty minutes after castration or control treatment, to one of four stimuli. In each group of 8 lambs, half were castrated during the trial (N=4), and the other lambs (N=4) were handled, but left intact. One of four stimuli; a cardboard box, a dog, a goat and a tape recorder playing the sound of a dog barking was presented to the lambs, or the lambs and their dams in an observation pen. The behaviour of the lambs was recorded for thirty minutes after treatment (castration, control), and then analysed.

3.2.2 Animals

In October 2003, lambs (N=384) aged between one and six weeks of age were subjected to routine castration or control treatment over four days. The animals were farmed at Tuapaka, a Massey University farm. Tuapaka is a hill country sheep and beef farm, situated on the outskirts of Palmerston North. The lambs used on day one were the offspring of Suffolk/Texel cross rams and Romney ewes, and on day two, lambs were the offspring of Texel rams and Romney/Finn cross ewes. The lambs used on days three and four were a mixture of either Suffolk/Texel/Romney cross or Texel/Romney/Finn cross-bred lambs. Each morning, ninety-six lambs and their ewes were mustered off pasture using dogs and penned together in covered yards. Lambs and ewes were penned in groups of about twenty-five lambs and ewes. No twin lambs were used in this study.

For the lamb only experiments, at the start of a trial, ewes and lambs were moved into a large pen, lambs were caught, allocated to a treatment, castrated or handled, and then placed in an observation pen in groups of eight lambs (Table 3.1). When the lambs and their ewes were used, at the start of a trial, groups of eight lambs and their respective eight ewes (Table 3.2) were herded into the observation pen. Lambs were then caught within this pen, allocated to their respective treatment, castrated or handled, and then returned to the observation pen. Castration was by the rubber ring method.

Table 3.1 The number of lambs without their dams, allocated to each treatment and stimulus over the first two days of the study period.

Lamb Stimuli	Number of lambs used in each treatment	
Stimuli	**Castrated**	**Control**
Dog	24	24
Barking Tape	24	24
Cardboard Box	24	24
Goat	24	24
TOTAL	**192 Lambs**	

Table 3.2 The number of lambs and ewes allocated to each treatment and stimulus over days three and four of the study period.

Lamb Stimuli	Number of lambs and ewes used in each treatment	
Stimuli	**Castrated**	**Control**
Dog	24 Lambs + 24 Ewes	24 Lambs + 24 Ewes
Barking Tape	24 Lambs + 24 Ewes	24 Lambs + 24 Ewes
Cardboard Box	24 Lambs + 24 Ewes	24 Lambs + 24 Ewes
Goat	24 Lambs + 24 Ewes	24 Lambs + 24 Ewes
TOTAL	**192 Lambs + 192 Ewes**	

Before being placed in to the observation pen (or returned to the observation pen, as was the case in the lambs and their ewes experiment), each lamb was picked up and either castrated or left intact, and marked with an individual spray painted symbol, on the withers for control lambs, or the rump for castrated lambs. This enabled individual lambs to be identified, and a distinction could also be made between castrated and control lambs (Figure 3.1). The stimulus was then placed in the observation pen.

Figure 3.1 Spray-painting the individual symbols on the lambs back following allocation of treatment.

Figure 3.2 The experimental pen.

The study was carried out between 8 am and 2 pm over the four days. After the thirty minute period was over, the animals were moved out of the observation pens. The stimulus was replaced, and the next lambs were picked up and the process was repeated. Groups of eight lambs were tested once, being exposed to only one of the four stimuli. At the end of the study, all the control lambs were castrated by rubber ring and all the lambs were tail docked with a docking iron and returned with their dams to a paddock. Due to a shortage of male lambs, some control lambs were ewe lambs to make up numbers, however over 90% of the control lambs were male.

The ewes in this study would have had some regular contact with the farmer and his dogs for all of their lives, e.g. moved between paddocks, shearing, drenching, whilst this would have been the lambs first intimate contact with dogs. However, both the lambs and their ewes would have had little experience with cardboard boxes and goats, and then only visual contact.

The use of all animals and procedures for this experiment was approved by the Massey University Animal Ethics Committee. Reference number 03/11 (Appendix 7.3).

3.2.3 Observation Pen

The observation pens were situated within a set of covered sheep yards. There were two pens (Figure 3.2), Pen A and Pen B. The two pens were side by side. Each pen measured 4.5 m x 3 m and had a dirt floor. The walls of each pen were covered in black plastic, so that the lambs could not see outside the pen. Observations began as soon as each lamb was placed in the observation pen. Observations thus began within 1 minute of the treatments being performed. The behaviours of the lambs during the castration or handling were not recorded.

A surveillance type camera with a 3.5-8.0mm F1.4 lens was positioned above each pen (Figures 3.3 and 3.4) at an angle so that that the entire pen was in view. This angle allowed the observer to distinguish between sitting and standing postures. Each camera was connected to a Panasonic AG6200 video recorder set to record the behaviour of the lambs on to a video-tape, for thirty minutes following castration or handling.

Human influence was controlled as much as possible in this experiment, by the provision of black plastic surrounding the pens. This reduced the amount of visual access the lambs and sheep had to humans, however humans could still be seen.

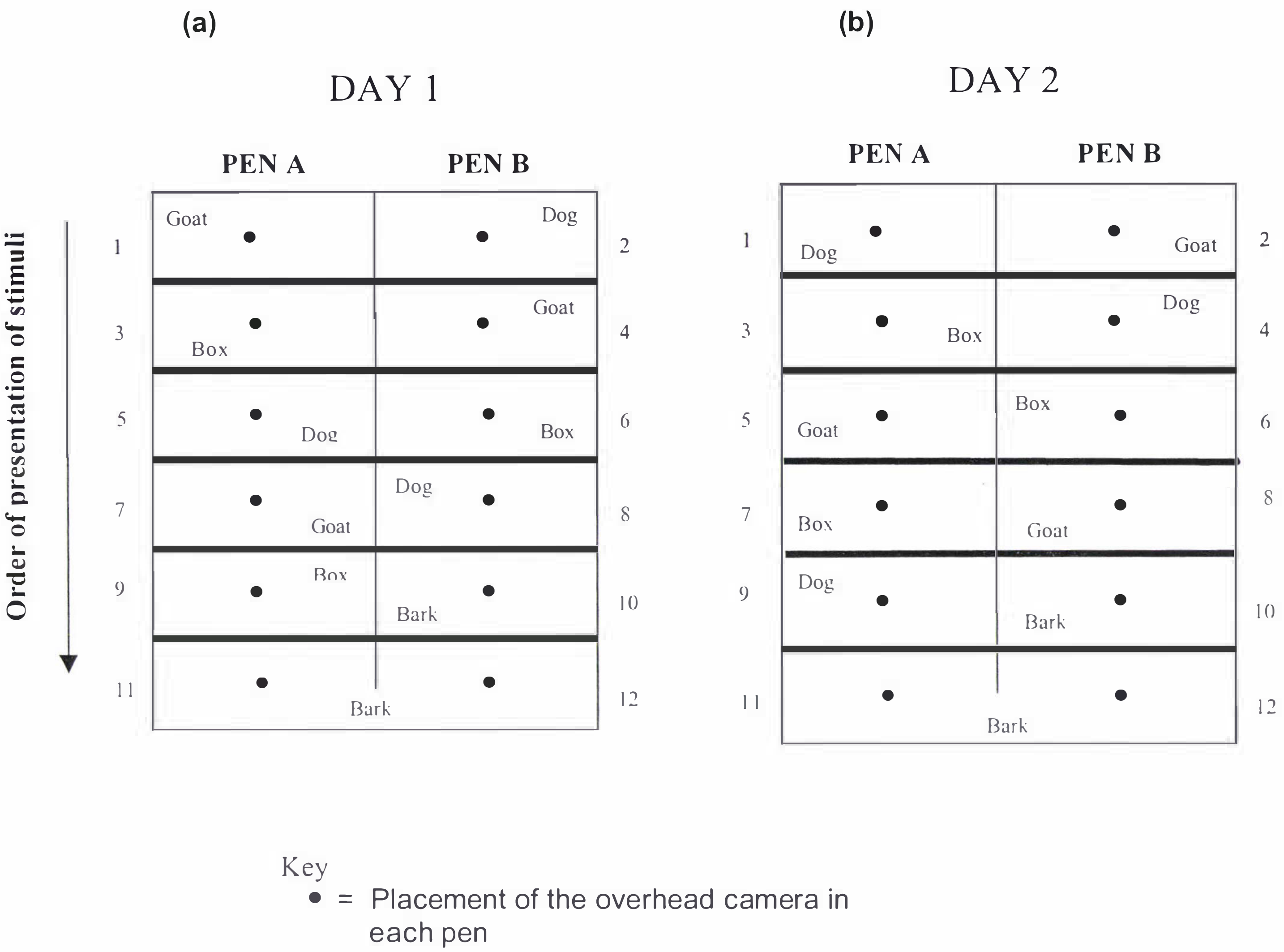
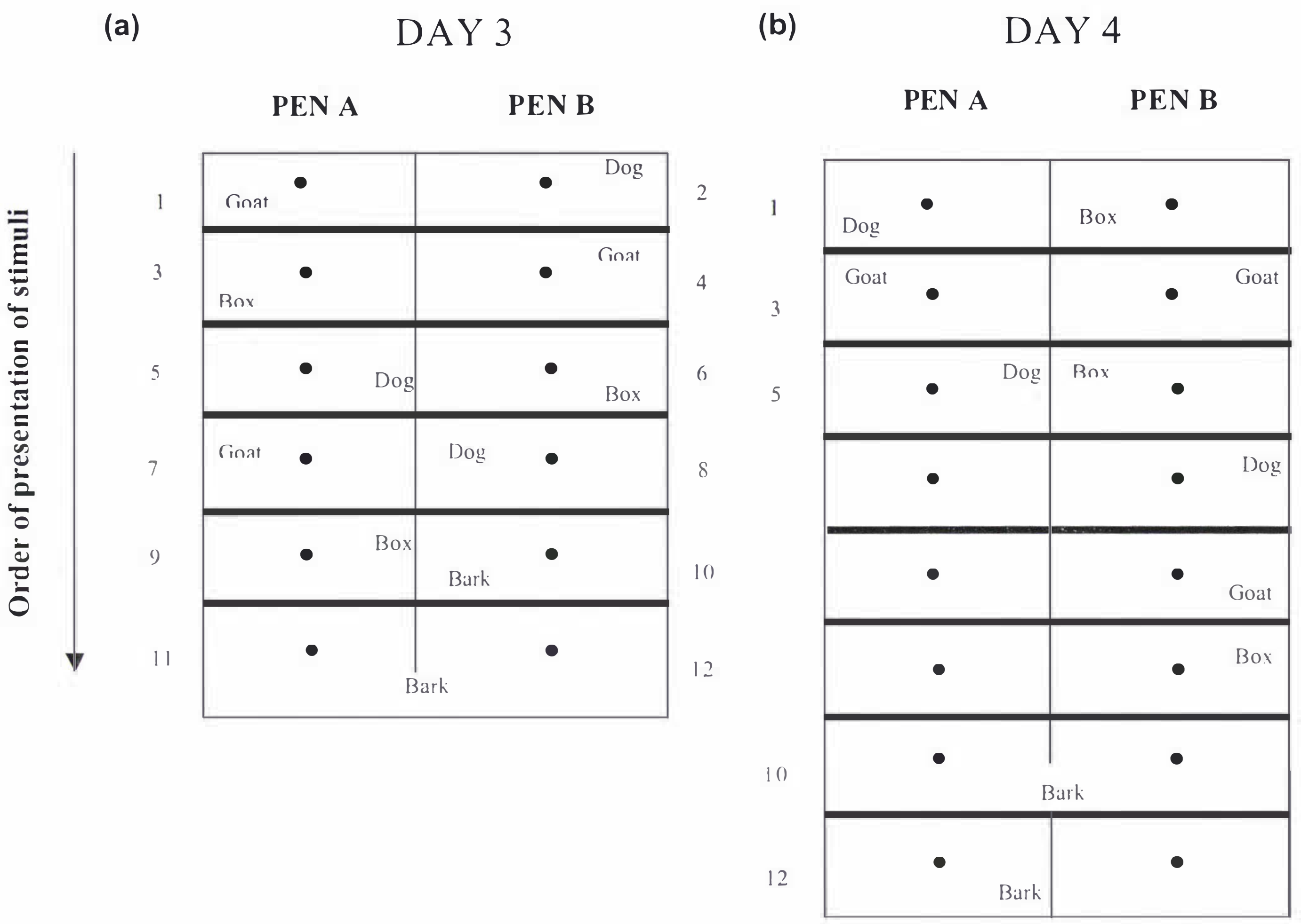


Figure 3.3 The order of presentation and the position of each of the four stimuli, in Pens A and B. The numbers down the sides of the diagram represent the order in which the trials were carried out in each of the two pens, over the day.



Key
• = Placement of the overhead camera in each pen

Figure 3.4 The position of each of the four stimuli and their order of presentation, in Pens A and B. The numbers down the sides of the diagram represent the order in which the trials were carried out in each pen.

3.2.4 Treatments

Lambs were randomly assigned to a test day and treatment, and were allocated to one of two treatments; castrated or control handling. One person caught and held the lamb on the pen wall, while a second person (always the same person) applied the treatment (Figure 3.5). Following treatment, lambs were immediately placed in the observation pen. The entire treatment procedure took approximately 1 minute in total for all eight lambs (four castrated, four controls) to be treated. Within groups, each treatment was given to four lambs. The age of the lambs was balanced across treatments and all rubber rings were taken from the same box.

3.2.4.1 Castration with rubber rings (C)

A latex rubber ring (Allflex New Zealand, Palmerston North): outer diameter, 14.8 mm, S.D., ± 0.1; inner diameter, 5.0 mm, S.D., ± 0.1, was applied to the neck of the scrotum, using an Elastrator (Elastrator Ltd, Blenheim) to stretch the rubber ring (Figure 3.5). Both testes were distal to the ring and the teats were proximal.

3.2.4.2 Handled Control (NC)

Lambs were handled and their scrotum palpated, as if they were being castrated, but they were left intact.

Figure 3.5 Castrating a lamb using an elastrator to apply the rubber ring.

3.2.5 Stimuli

During a trial, one of four stimuli was presented to the eight lambs for thirty minutes after treatment and their behaviour was videotaped for this period of time. Twelve tests were performed each day, and each stimulus was used three times each day (Tables 3.1 and 3.2). Stimuli were randomly allocated to pens using a balanced design to reduce the residual effects of the treatments. Stimuli were randomly allocated to an area of each pen (Figure 3.3 and 3.4). All stimuli were unfamiliar to the lambs.

3.2.5.1 Dog

A sixth month old, well bred, female huntaway dog was tied in one corner of the observation pen (Figure 3.6). The dog was commanded not to bark throughout the trial period. When the dog was not involved in an experiment, it was kept tied up, silent and out of view in an adjacent pen. The same dog was used for the four-day trial.

Figure 3.6 The experimental pen showing the lambs, and the dog as the stimulus (Far right).

3.2.5.2 Barking Tape

A tape recorder playing the sound of a dog barking was placed at one end of the observation pen. The tape used was the recording of a trained heading dog barking. The same tape was used throughout the experiment. The trials involving the use of the barking tape were always carried out in the afternoons. This was so that the lambs used in pens with the other three stimuli were not affected by the dog barking tape, as they had all been carried out in the morning.

3.2.5.3 Cardboard box

A large white cardboard box was placed at one end of the observation pen (Figure 3.7). The dimensions of the box were 60cm height x 60cm width x 40cm depth. The box control was used as a novel object with no biological significance to the sheep (Beausoleil et al., 2005). The same cardboard box was used for the four-day trial.

Figure 3.7 Lambs and their ewes in the experimental pen with the cardboard box stimulus.

3.2.5.4 Goat

Two white, four year old, male angora goats, sourced from a local farmer were used in this experiment. Only one goat was used at a time. The goat was tied in one corner of the observation pen during an experiment (Figure 3.8). Each goat was tied in an adjacent pen out of view, and was silent, when not involved in an experiment.

Figure 3.8 Lambs and their ewes in the experimental pen with the goat as the stimulus (Front left).

3.2.6 Video Reading

Each of the videos used to tape the four-day experimental period were viewed on a television screen, and data were collected and recorded. The clock on the screen of the video player provided the time increments for each experiment. The time when each lamb was placed into the pen was recorded. Data collection was carried out for thirty minutes following all eight lambs being placed in the pen.

3.2.7 Data Gathering

Initially only one castrated and one control lamb was analysed from each video. From these initial observations, it was decided that the behaviour of all eight lambs (four castrated, and four controls) in each pen would be observed and recorded to obtain as much data as possible from the experiment.

3.2.8 Behaviours

The two behaviours that were monitored were restlessness, and the average time the lamb spent sitting. The data for each of these behaviours were collected from analysing the video tapes of the experiment.

3.2.8.1 Restlessness

This was scored as the number of times that a lamb stood up or lay down; with each time a lamb stood up or lay down being recorded (Lester *et al.*, 1996).

3.2.8.2 Average time spent sitting

Each time a lamb sat down, the time the lamb sat, as well as the corresponding time it stood up, was recorded. From this, the average length of time each lamb spent sitting could be calculated for the thirty-minute trial period.

3.2.9 Data Analysis

None of the behavioural data were normally distributed; so all data were transformed to approximate normality by taking the square root of raw data. A repeat measures analysis of variance of the behaviours in relation to the four treatments, bark, goat, dog and box was carried out. Also a repeat measures analysis of variance was used to test the effects of the pen, day and time of day and to test for variation between castrated and control lambs. Because the non-castrated lambs exhibited low restlessness scores, one was added to each raw data value, for both castrated and non-castrated lambs so that the data could be transformed, and therefore a comparison could be made between these results (Zar, 1999). All statistical tests were performed using SAS Version 8.0 (SAS Institute Inc., 1994).

Data from this study can be found in Appendix A (Figure 7.1).

3.3 Results

There were no major difficulties over the four days of the study. Presentation of the stimuli (box, goat, dog and barking tape) presented no problems. However, due to an insufficient number of males for full replication according to the experimental design, some controls used in this experiment were female lambs. In pens with insufficient numbers of male castrated lambs, numbers were made up with female lambs, but the behaviour of these female lambs was not recorded, and for statistical purposes these data were recorded as missing.

There was a significant difference between the restless behaviour of castrated and control animals as detailed in 3.3.1 below. There were no significant differences between stimuli. The presence of the ewes, however, had a significant effect on the restlessness behaviour exhibited by the lambs.

3.3.1 Restlessness

Castrated lambs had significantly higher restlessness scores (ANOVA, $F_{1, 82} = 563.27$, $P<0.0001$) than control lambs (Figure 3.9), with none of the control lambs achieving a score equal to the average restlessness of the castrated lambs. Castrated lambs on average stood up and sat down 26 times in the thirty minutes following treatment. In contrast, control lambs stood and sat 1.2 times (raw data).

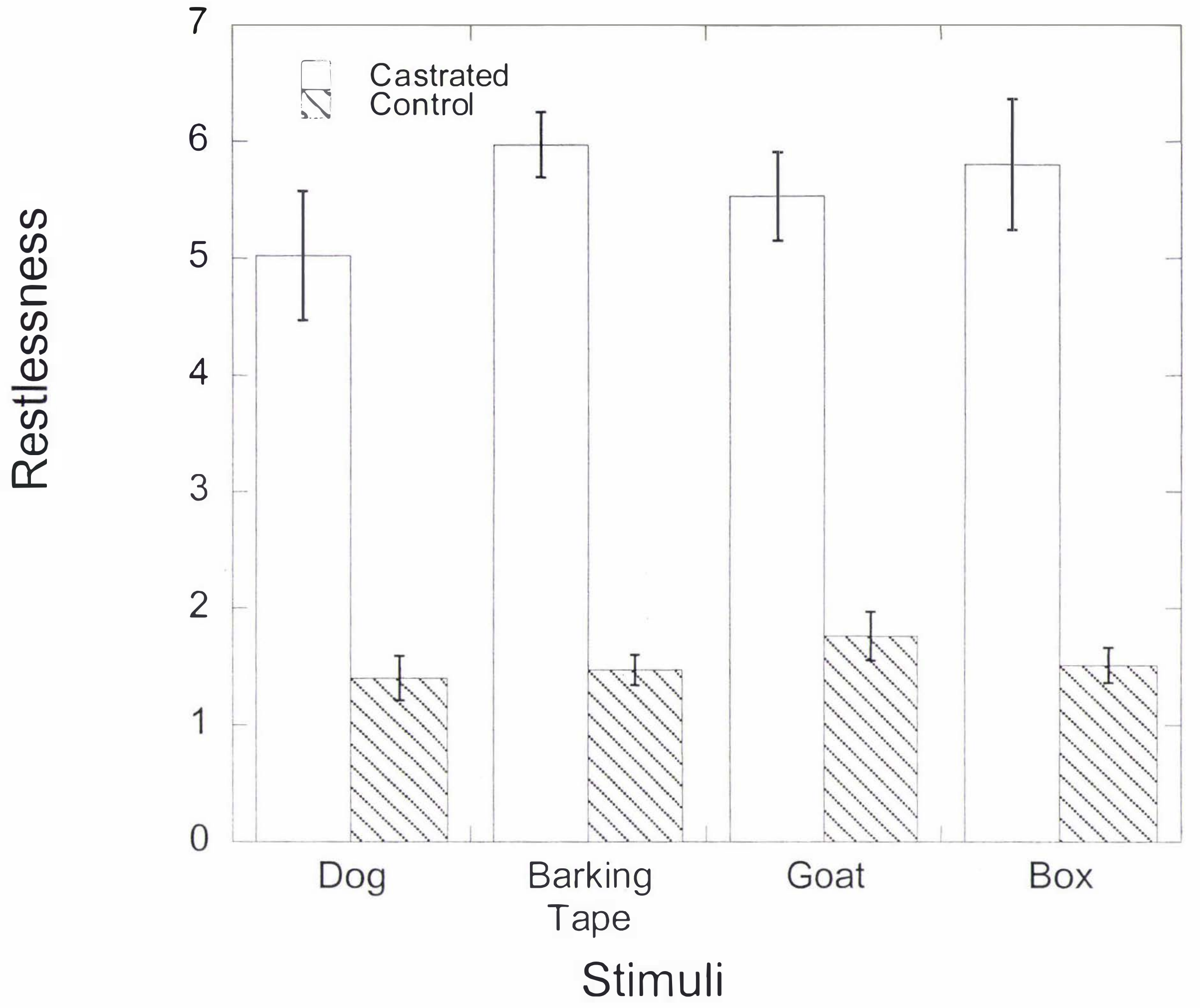


Figure 3.9

Mean restlessness behaviour exhibited by castrated and control lambs when their dam was absent, in the presence of each of the four stimuli (± S.E.M). Data were transformed by a square root transformation. Each time a lamb stood up or sat down was recorded as one instance of restlessness.

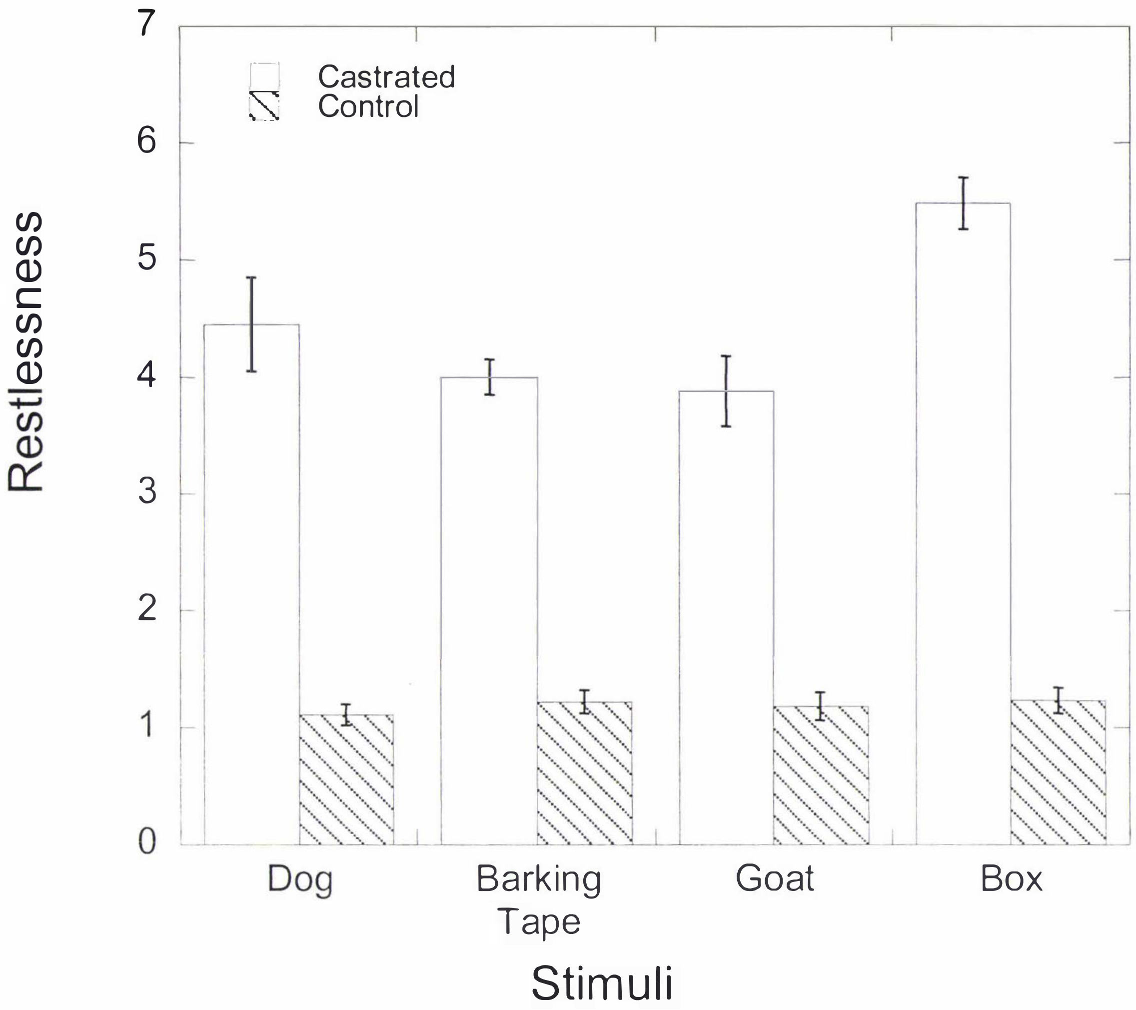


Figure 3.10

Mean restlessness behaviour exhibited by castrated and control lambs when their dam was present, in the presence of each of the four stimuli (± S.E.M). Data were transformed by a square root transformation. Each time a lamb stood up or sat down was recorded as one instance of restlessness.

There was no effect of stimulus (Box, Dog, Bark, Goat) amongst control lambs with and without their dams present (Figure 3.10), on the restless behaviour of the lambs (ANOVA, $F_{3,38}$= 0.74, N.S). In addition, there was no effect of stimulus on the restless behaviour of castrated lambs with or without their dam present (ANOVA, $F_{3,34}$ = 2.24, N.S).

There was no effect of pen on the behaviour of either castrated lambs (ANOVA, $F_{1, 34}$=0.07, N.S), or control lambs (ANOVA, $F_{1, 38}$ = 0, N.S).

There was, however, a significant effect of dam on the restlessness behaviour of both castrated lambs (ANOVA, $F_{1,34}$=16.58, P=0.0003) (Figure 3.11), and control lambs (ANOVA, $F_{1,38}$=11.12, P=0.0019) (Figure 3.12). Both castrated and control lambs were more restless when their dams were absent (Figures 3.11 & 3.12).

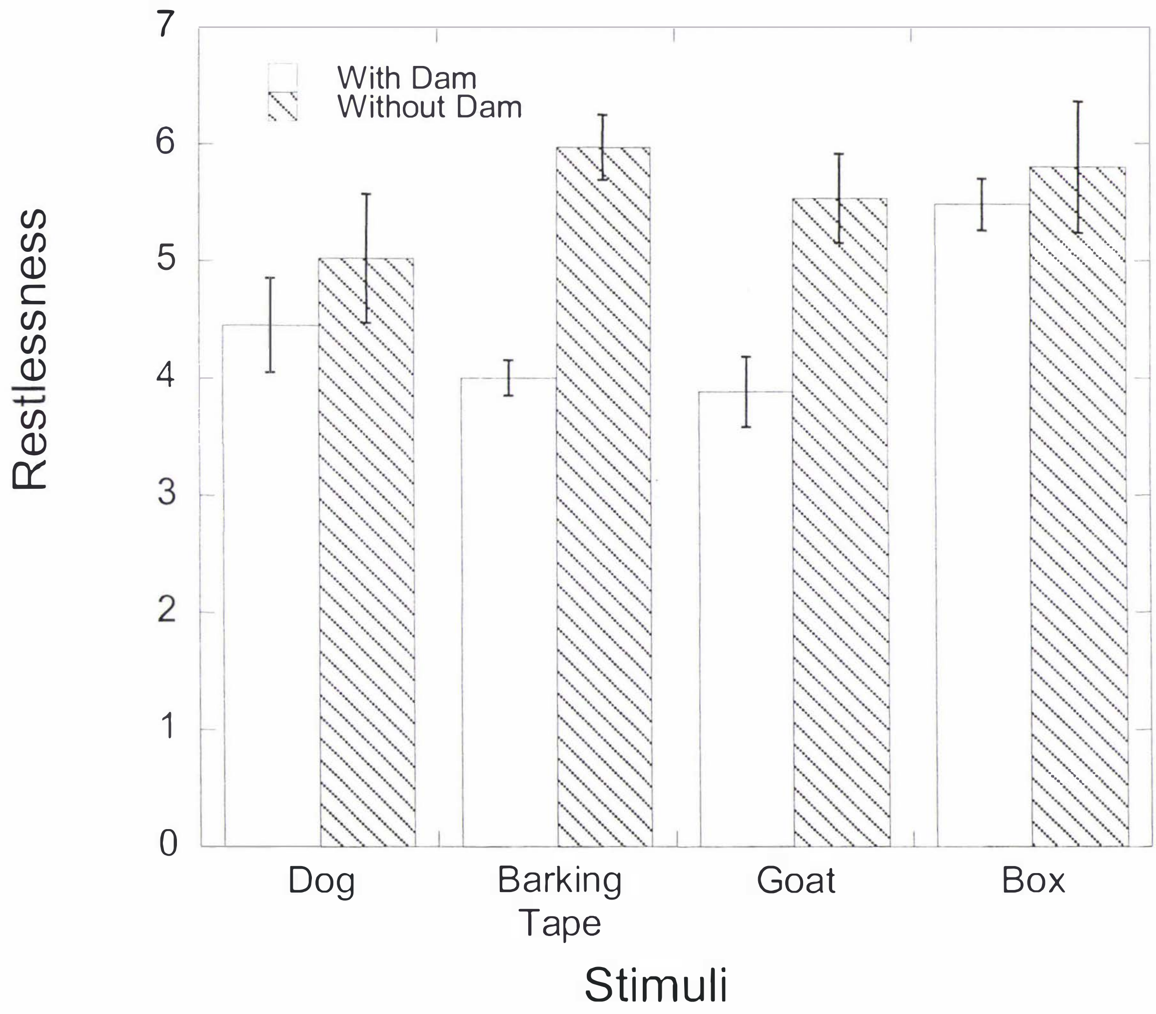


Figure 3.11

A comparison of the mean restlessness behaviour exhibited by castrated lambs when their dam was present and absent, in the presence of each of the four stimuli (± S.E.M). Data were transformed by a square root transformation. Each time a lamb stood up or sat down was recorded as one instance of restlessness.

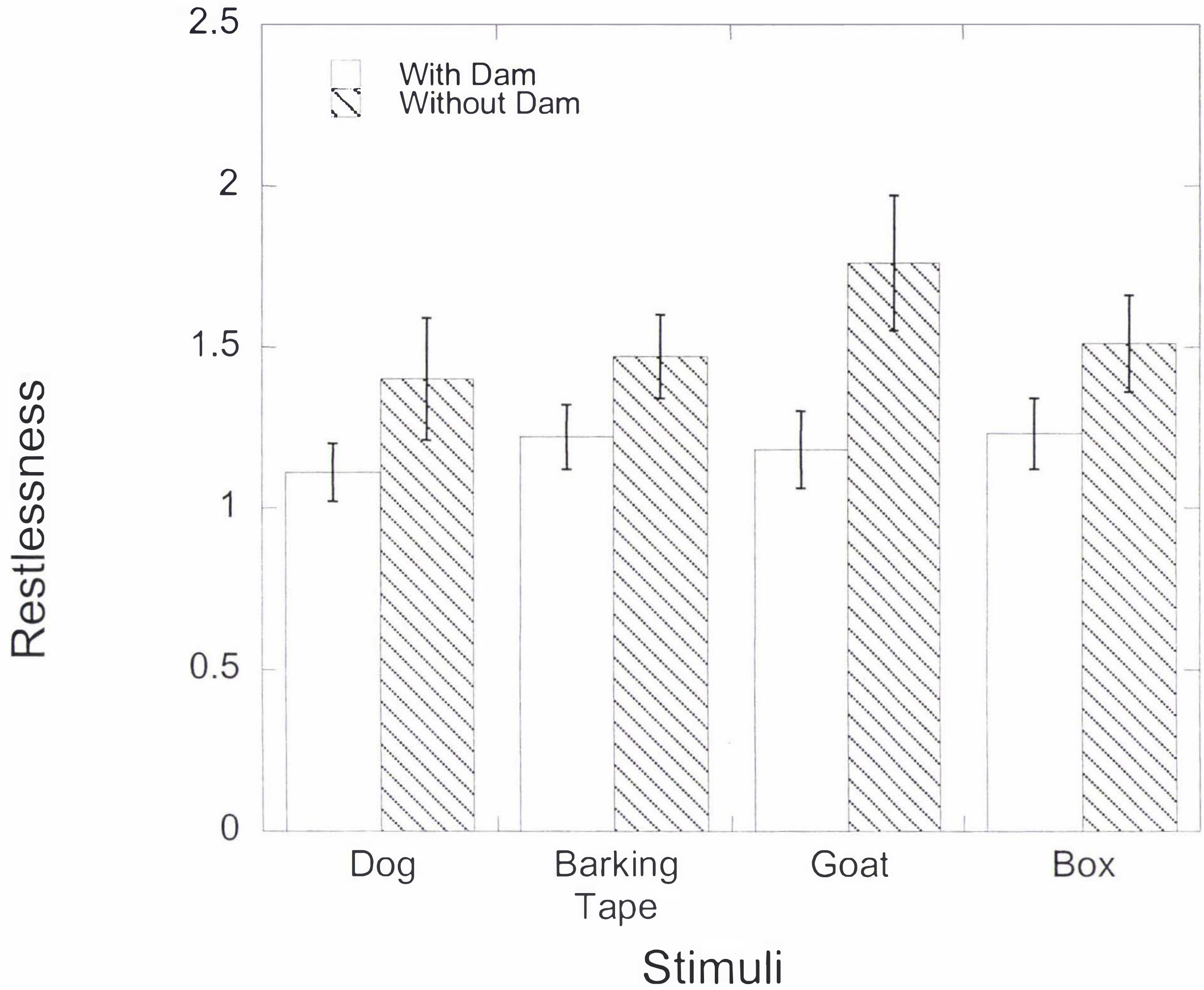


Figure 3.12

A comparison of the amount of restlessness behaviour exhibited by control lambs when their dam was present and absent, in the presence of each of the four stimuli (± S.E.M). Data were transformed by a square root transformation. Each time a lamb stood up or sat down was recorded as one instance of restlessness.

3.3.2 Average time spent sitting

A comparison of the lengths of time castrated lambs spent sitting in the presence or absence of their dam revealed that there was no effect of dam (ANOVA, $F_{1, 34}$=2.53, N.S) on the average time castrated lambs spent sitting, nor was there an effect of stimulus (ANOVA, $F_{3, 39}$=1.15, N.S), or pen (ANOVA, $F_{1,39}$= 2.43, N.S).

3.3.3 Time first sat

The time that castrated lambs first sat was affected by the presence of their dam (ANOVA, $F_{1, 26}$ =8.77, P=0.0065). On average, castrated lambs with their dam first sat after 3.68 minutes of being placed in the pen, whereas castrated lambs without their dams sat within 2.95 minutes of being placed in the pen.

3.4 Discussion

This study of the behaviour of lambs and by inference the pain caused by rubber ring castration and, the influence of the four stimuli on this behaviour, provided the following information.

Firstly, castration caused a significant increase in restlessness behaviour. As anticipated in the hypothesis, this increase was significantly greater than that of control lambs and indicates that castration is acutely painful.

Secondly, there was no significant difference in the restlessness behaviour of either control or castrated lambs in the presence of any of the four stimuli (box, goat, dog, bark). This suggests that the lambs were reacting more to the treatment, and to the novelty of the situation, than to the individual stimuli.

Thirdly, when the lambs' dams were present, restlessness behaviours were significantly reduced in both castrated and control lambs. In addition, when the ewes were present, the first incidence of restlessness (time first sat) for each lamb occurred after a longer period of time. This suggests that ewes influenced the behaviour of their lambs.

Restlessness was studied during the first thirty minutes after treatment and the scores ranged from low (<13) in control animals to high (>80) in castrated lambs. This is comparable to other studies, for example control and ring castrated lambs, in the first hour after treatment, had restlessness scores ranging between less than 8 to greater than 70 respectively (Dinniss et al., 1999). In a similar study, Dinniss et al. (1997) reported that lambs with a restless score less than eight, as seen in control animals, exhibited a small cortisol response. In comparison, lambs with restlessness scores above 40 were probably in severe pain as animals with these scores had a cortisol response which was significantly greater than that in control animals (Dinniss et al., 1997). There is a significant increase in the levels of restlessness behaviour exhibited by lambs following rubber ring castration (Lester et al., 1996), and these levels of activity peak within the first thirty minutes after treatment (Lester et al., 1996; Molony et al., 2002). Restlessness is therefore regarded as a sensitive indicator specific to a particular noxious stimulus, namely ischaemic pain (Dinniss et al., 1999).

The finding that lambs did not behave differently in the presence of any of the four stimuli suggests that the test subjects were reacting to the novelty of the stimuli and possibly viewed all four stimuli as potential threats because of this novelty. In addition, it is possible the lambs were reacting to the treatment (castration or control), and that these were more important than the stimuli. It is possible that the lambs were reacting to the novelty of both the situation and the stimuli, and that they had not learned to make negative associations with any of the four stimuli. Porter & Bouissou (1999) found that lambs did not respond with aversion to images of dogs as adult ewes did, suggesting that the lambs had not yet had enough experience with dogs to link unpleasant associations with them.

The reduction of the lambs' restlessness behaviour in the presence of their dams suggests two things. Either lambs were reacting to a shortage of space in the pen when their ewes were present, or the ewe was cueing the lamb in some way to the presence of the stimulus. Lambs of this age still rely heavily on protection from their mother against threats because juvenile prey animals are vulnerable to a wider range of predators than adults, as a result of their small size (FitzGibbon, 1990). It would therefore be advantageous for them to closely follow the actions of their mother in dangerous situations. This is an interesting finding; however, future studies are required to make a distinction between whether the animals were reacting to a lack of space or whether the ewes were actually cueing their lambs.

The use of live animals as stimuli in this experiment may be problematic, as inconsistencies in stimulus behaviour can influence the responses of the test lambs. For example, the goats were quite aggressive towards test lambs, including bunting some of the lambs, whilst the dog generally appeared bored and indifferent. However, in order to determine the behavioural responses of sheep to a range of 'real' stimuli, the use of live animals was deemed most appropriate. Similar observations were documented by Beausoleil et al. (2005). Beausoleil et al. (2005) speculated that the sheep regarded the goat as a strange conspecific; this is supported by the behaviour of the lambs in this study. Although not quantified, lambs appeared to spend more time closer to the goat and cardboard box, than to the predator stimuli (dog, barking tape). Measuring walking by the lambs in relation to the position of the stimulus may have provided some interesting results in this study. It has been shown that sheep will maintain a close distance to a cardboard box, whilst maintaining a longer distance from a dog when given the choice (Beausoleil et al., 2005). In future this should be measured, as this may be a more sensitive indicator of how the test subjects viewed each of the four stimuli.

In this study, in contrast to Beausoleil et al. (2005), but supported by others, (Torres-Hernandez & Hohenboken, 1979; Romeyer & Bouissou, 1992) vocalisation appeared to be suppressed in lambs, in the presence of all stimuli (personal observation).

Odours were difficult to control for in this study. These odours could have been from the stimulus that was previously in the pen, or odours from the stimulus being used in the adjacent pen. However, this variable was controlled for by making sure the stimuli were randomly allocated to each pen, ensuring that the same two stimuli did not always follow each other in the order of their presentation to the lambs. Furthermore having an observer that was 'blind' to the experiment may have been a useful tool, especially when observing between castrated and control lambs, however this was not practical in this study, and it could be argued that the experimentally 'blind' observer would very quickly work out which animals were castrated based on the amounts of restlessness exhibited, making the necessity of a 'blind' observer somewhat redundant.

The use of female lambs as control animals was not intended, but due to insufficient numbers of ram lambs, females were used. It is possible that female lambs behave differently than rams, which may therefore affect the validity of the results from this study. However, based on the results, a slight difference between the behaviour of male and female lambs would not have changed the strong significant difference between castrated and control lambs. Nevertheless, the difference between the behaviour of male and female lambs should be examined in future studies to verify this.

This research has only studied the castration of ram lambs, further research should be extended to include ewe lambs by using tail docking as an alternative to castration, as a painful stimulus for example, and also on other breeds of sheep as behavioural responses to pain, stress and fear can differ between breeds (Vierin & Bouissou, 1992; Hansen et al., 2001).

These results indicate that lambs between 1-6 weeks of age have not yet learned negative associations with dogs due to a lack of experience. These results suggest however that at this age, lambs are relying on their mother's experience, and direction. However because ali of the stimuli were 'novel' to the lambs, it may be the novelty that the lambs are reacting to, more than the potential threat. It therefore may have been of more value to have tested the lambs with a control of "no stimulus" to control for this possible variable, novelty. Future studies should therefore consider this.

4 The Effect of Predator Presence on the Mechanical Pressure Thresholds of Ewes

4.1 Introduction

Nociceptive threshold tests measure the responsiveness of animals to noxious stimuli. These techniques record changes in an animal's nociceptive threshold associated with physical injury or surgical trauma (Whay et al., 1997) and have been used to study acute and chronic pain in animals (Ley et al., 1989; Lascelles et al., 1995; Welsh & Nolan, 1995). Following injury or trauma, individuals demonstrate an increased sensitivity to noxious stimuli, by responding to a stimulus at a lower level than seen in a healthy subject. This decrease in nociceptive threshold is a demonstration of hyperalgesia, an increased sensitivity to pain (Whay et al., 1997). Nociceptive thresholds have been found to be significantly lower in sheep with footrot (Ley *et al*., 1995) and lame dairy cattle (Whay et al., 1997) than healthy conspecifics. Furthermore, they have been used to test the analgesic effect of drugs (Ley, Waterman, & Livingston, 1991; Waterman, Livingston, & Amin, 1991). However, these studies have not examined whether the response of animals to a noxious pressure stimulus, and by inference pain, will change in the presence of a predator. This experiment studied the response of healthy sheep, to a painful mechanical pressure device, in the presence of a predator.

If a property of an individual that reduces it chances of being taken by a predator is favoured by natural selection as suggested by Ridley (1995), then it can be assumed that showing pain which would disadvantage prey animals in the presence of a predator would be selected against. Therefore, sheep should increase their response thresholds to a painful stimulus in the presence of a predator in an attempt to reduce their chances of being taken by a predator.

The objective of this experiment was to determine if sheep, subjected to a mechanical pressure device would alter their behaviour in the presence of specific stimuli (dog, goat, a tape recorder playing the sound of a drum beat and a tape recorder playing the sound of a dog barking), and more specifically, whether sheep would increase their response thresholds in the presence of a predator, in an effort to hide the pain being experienced from a potential predator.

The hypothesis to be tested was that sheep would increase their response thresholds to a painful pressure stimulus significantly more in the presence of a predator (dog and dog barking tape), then when an arbitrary noise (drum beat tape) was played, or a goat was present.

4.2 Methods and Materials

4.2.1 Introduction

This study investigated the effect of various stimuli (dog, barking tape, goat, drum beat) on the thresholds to mechanical stimulation in sheep. Sixteen ewes were trained to stand in a metabolic crate with a mechanical pressure device fastened around their back right leg. Five baseline mechanical pressure threshold readings were taken with no stimuli present, and then each of four stimuli; a dog, a goat, a tape recorder playing the noise of a dog barking and the noise of a drum beat tape were presented to the ewes over a four day period; one stimulus per sheep, per day. The threshold values were recorded and analysed.

4.2.2 Animals

In December 2003, mechanical nociceptive stimulation was used to test the response of mixed aged Romney ewes (N=16) to the presence of a predator. The ewes had been raised together since weaning on a Massey University farm; the large animal teaching unit (LATU). These sheep would have had regular contact with a farmer and farm dogs for most of their lives, e.g. moved between paddocks, shearing, drenching. However they would have had little experience with goats, and then only visual contact. Four additional sheep from the same mob were used as buddy sheep.

All of the sheep were familiarised with the yards, races and test area before testing, in order to reduce the confounding effects of novelty on the behavioural responses of the sheep. For the two days prior to the experiment, the test sheep were moved through the facility, and were trained to stand in the metabolic crate, and to accept their back legs being handled. The day before trials began; each sheep was individually marked with a spray painted number.

The ewes were taken off pasture and penned together outside the testing building each morning of the four-day study. On the morning of each trial, the test sheep were drafted, as a group, out of the flock. Four buddy sheep were randomly selected from the remaining flock and placed inside in a pen, which was separated from the test sheep by a gate, to reduce the effects of isolation stress on the test sheep (Figure 4.1). Immediately before a trial, individual test ewes were gently pushed along a race, by a handler, into the building and guided into a metabolic crate. Following a trial, the sheep were moved into a post-test pen outside. At the end of each day, all sheep were put out to pasture together. All tests were carried out between 7 am and 7 pm. The use of all animals and procedures for this experiment was approved by the Massey University Animal Ethics Committee. Reference number 03/122 (Appendix 7.3).

4.2.3 Observation pen

The observation area was a room, situated within a large concrete floored building. By holding the test animals within this room, the sound of the auditory stimuli (drum beat and dog barking) could not be heard by the remaining test sheep outside, so they had no previous experience with any of the stimuli. The test area measured 3.85 x 4.95 metres and contained portable metal yards set up into three pens (Figure 4.1). These were; a pen for the stimulus (4.0 m x 1.5 m); this had a door leading in to the main room, where the stimuli were held, a pen for the buddy sheep (1.5 m x 2 m), and a metabolic crate (0.9 m x 0.56 m) for the test sheep. The latter two pens could be accessed from the outside yards. The test sheep had visual and auditory access (and limited physical access) to the buddy sheep at all times during the test (never visually isolated), as well as visual and auditory access to the stimulus (Figure 4.1). Whilst the buddy sheep pen and the stimulus pen were fastened with metal gates, the metabolic crate for the test sheep was fastened at the rear with a metal chain and clip (Figures 4.1 & 4.2). This enabled the observer easy access to the test sheep's back legs, to apply the mechanical pressure device.

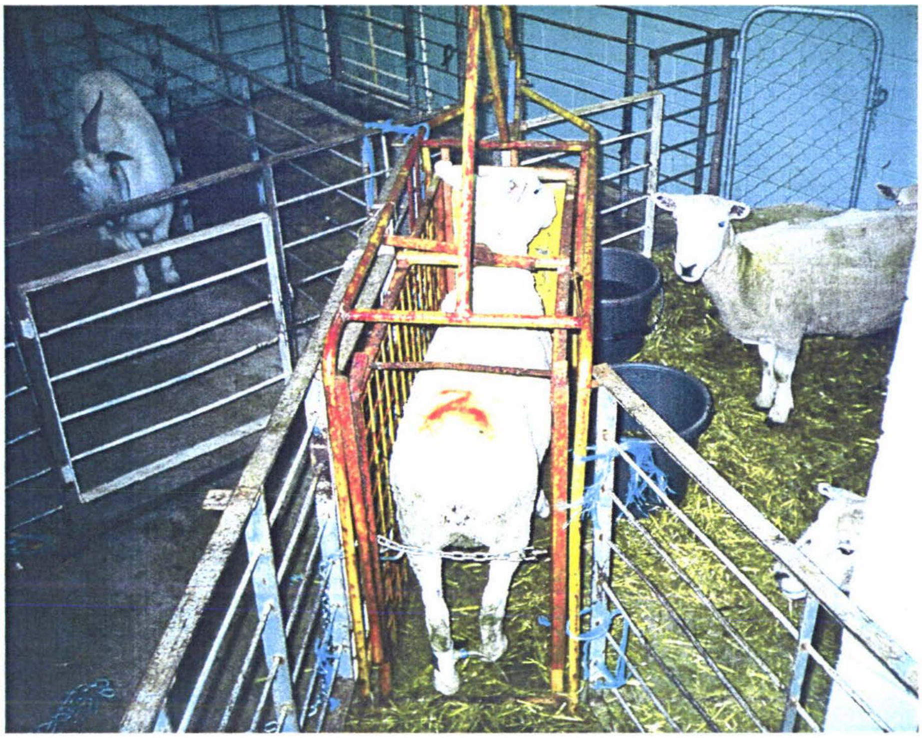


Figure 4.1.

The experimental set up. On the left is the stimulus pen, with the goat as the stimulus. In the metabolic crate is the test sheep, with the mechanical pressure device attached to its left hind leg, and to the right are the buddy sheep.

Figure 4.2.

The mechanical pressure device attached to the sheep's hind left leg, and the chain used to secure the sheep inside the metabolic crate.

4.2.4 Treatments

Each animal was placed in the metabolic crate beside a pen containing four buddy sheep, to reduce the effects of stress. The threshold device was fastened around the test sheep's leg (Figure 4.2) and five minutes was allowed for the animal to acclimatise before the first baseline pressure reading was taken. The threshold was assessed on the animal's back left leg by using a device, which pneumatically pushed a blunt pinhead against the skin of the leg. The threshold response to this device was recorded when there was a clearly defined lifting of the leg. This technique and the device used in this study are similar to that described by Nolan, Livingston, Morris, and Waterman (1987) and Chambers, Livingston, and Waterman (1990). The force applied to the pin was measured on a pressure gauge to give a threshold value in pounds per square inch (lb/in^2). Following the lifting of the sheep's leg, the threshold value was recorded. Four or five baseline threshold values were recorded at three-minute intervals until at least four consistent readings were obtained, and these were recorded. The stimulus was then placed in an adjacent pen (Figure 4.1) where it could be viewed and heard by both the test and the buddy sheep. A single stimulus threshold reading was then taken after three minutes of the stimulus being place in the pen, and recorded. Each sheep was tested only once each day.

4.2.5 Stimuli

During each trial, one of four stimuli was presented to the ewes for three minutes and then a pressure stimulus reading was taken. Each stimulus was presented four times on each day, to four different sheep, with the order of presentation being randomised for each day, based on a residual effects Graeco-Latin square experimental design. Four consecutive days of testing allowed each of the sixteen sheep to be presented with each of the four stimuli once, and to be exposed to only one of the four stimuli on any one day. All stimuli were placed in the same area of the pen (Figure 4.1), and were unfamiliar to the ewes.

4.2.5.1 Dog

A six month old, well-bred, female huntaway dog was tied in one corner of the observation pen. The dog was commanded not to bark throughout the trial period. When the dog was not involved in an experiment, it was kept tied up, and out of view in an adjacent pen. The same dog was used for the four days of this study.

4.2.5.2 Barking Tape

A tape recorder playing the sound of a dog barking was placed at one end of the observation pen. The recording was of a trained heading dog barking.

4.2.5.3 Drum Tape

A tape recorder playing the sound of a drum beat was placed at one end of the observation pen. This stimulus was used as a control novel sound.

4.2.5.4 Goat

A white, four year old, male angora goat, sourced from a local farmer was used in this experiment. The goat was tied to the side of the metal stimulus pen during an experiment (Figure 4.1), and was tied in an adjacent pen out of view, when not involved in an experiment.

4.2.6 Data Gathering

Two people assisted with data gathering, these were; a recorder and a reporter. The recorder increased the pressure on the mechanical device until the reporter gave the verbal cue to stop. At this cue, the recorder noted the pressure reading on the equipment and recorded the data, and released the pressure device from the leg of the sheep. A third person assisted with the introduction of the stimuli, allowing the recorder and the reporter to remain stationary. At least five values were obtained from each sheep on each of the four days of the trial; at least four consecutive baseline values, and one stimulus value.

4.2.7 Data Analysis

A single baseline value for each sheep, for each day of the trial was obtained by averaging four baseline values taken before the stimulus for that day was shown to the sheep. Therefore each of the 16 sheep in the trial had four averaged baselines, one recorded for each day of the four-day trial. This averaged baseline value was then subtracted from the corresponding stimulus value obtained for each sheep. This gave a single value for each sheep, for each stimulus, representing the difference in pressure threshold in response to the stimuli. None of the mechanical pressure threshold difference data were normally distributed, so all data were transformed to approximate normality by adding ten to each data value and then taking the square root of these new values. An analysis of variance was then performed on these transformed data in relation to the four treatments, bark, goat, dog and noise. An analysis of variance was also used to test the effect of day on the response of the sheep. All statistical tests were performed using SAS Version 8.0 (SAS Institute Inc., 1994).

Data from this study can be found in Appendix B (Figure 7.2).

4.3 Results

The mean pressure threshold in the absence of stimuli (referred to as the baseline value) recorded before the sheep were shown the dog was 5.9 pounds per square inch (lb/in^2), 6.76 lb/in^2 before the dog barking tape, 5.81 lb/in^2 before being played the drum tape recording and 6.168 lb/in^2, before being shown the goat. In comparison, when shown the stimulus, the mean pressure threshold in the presence of the dog was 8.3 lb/in^2, 6.4 lb/in^2 in the presence of the goat, 6.3 lb/in^2 in the presence of the drum beat tape and 6.0 lb/in^2 in the presence of the dog barking tape. Sheep mechanical pressure thresholds were affected significantly by the presence of the four stimuli (dog, goat, noise, bark) (ANOVA, $F_{3,12}$ = 6.81, P=0.0062) (Figure 4.3). Furthermore, the analysis of variance revealed a significant effect of animal (ANOVA, $F_{15,12}$ = 2.66, P=0.0473), which suggests that some individuals had a consistently higher or lower pain threshold overall, irrespective of the stimulus. In this experiment there was no effect of day on the pressure thresholds of sheep (ANOVA, $F_{1,11}$ = 0.06, N.S).

As predicted, the difference between the mean of the baseline values and the stimulus value showed the response of sheep to the dog (2.04 ± 0.78) (mean ± s.e) was greater than the response to the goat (0.28 ± 0.51) (t=1.99, P<0.05, df=39, one tailed t-test). In addition, sheep mechanical pressure thresholds were significantly higher in the presence of a dog than when presented the sound of a dog barking (Figure 4.3).

When sheep pressure thresholds in response to the four stimuli were compared to their baseline values and averaged, sheep pressure thresholds increased in response to the presence of the dog and goat (results presented above) and the drum beat by 0.51 lb/in^2. However, in contrast, when presented the sound of a dog barking, sheep pressure thresholds decreased by 0.81 lb/in^2 on average.

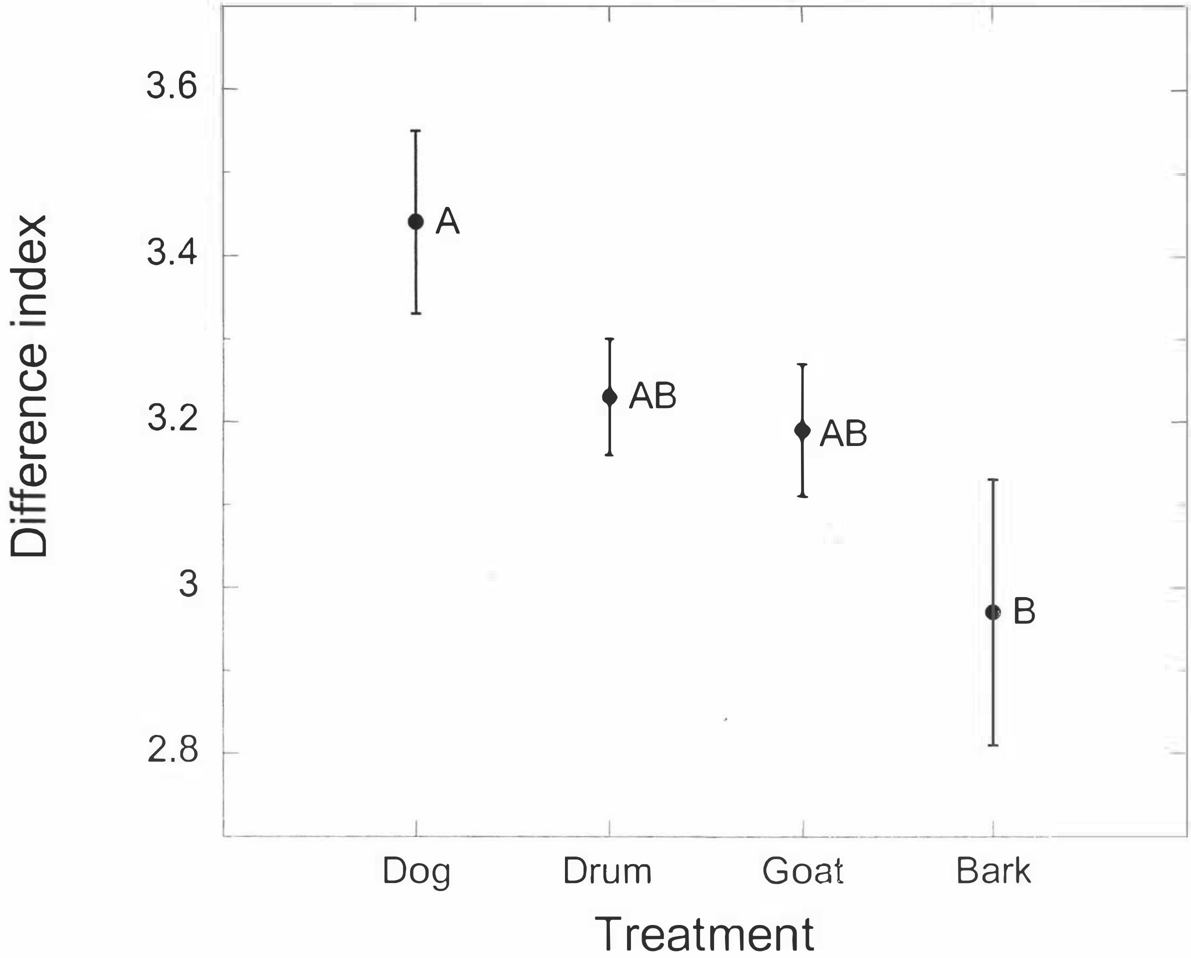


Figure 4.3. The mean pressure threshold response of sheep to each of the four treatments (± S.E.M). The difference index is the difference between the pressure threshold value obtained in the presence of the stimulus minus the average of the four baseline pressure threshold values. The data presented here were transformed using a square root transformation to approximate normality. Means with different letters are significantly different at $P<0.05$.

4.4 Discussion

This study of the mechanical pain threshold responses and by inference the pain caused in sheep in the presence of four stimuli, provided the following information.

Firstly, all four stimuli significantly influenced the mechanical pressure threshold of the sheep. This infers that there were differences in the way the sheep were responding to each of the four stimuli, in relation to the pressure stimulus.

Secondly, sheep withstood significantly more pressure in the presence of the dog than when in the presence of the goat. This suggests that the sheep found the dog more aversive than the goat.

Thirdly, sheep withstood significantly more pressure in the presence of the dog than when in the presence of the dog barking tape. This suggests that the sheep found the dog more aversive than the tape recording of a dog barking.

It was hypothesised that sheep would withstand more pressure in the presence of the two predator stimuli; the dog and the dog barking tape recording. The behaviour of the sheep in response to the presence of the dog, and the goat suggests that the sheep were able to recognise the dog as a predator and react accordingly. These results fit with the findings of other studies. Beausoleil et al. (2005) found sheep showed more aversion towards a dog than a goat when placed in an arena test. In addition, dogs are known predators of sheep (e.g. Bogess et al., 1978; Robel et al., 1981; Schaefer et al., 1981 cited in Beausoleil et al., 2005; Hansen et al., 2001). Goats however, are not predators and evidence suggests that they are recognised, along with conspecifics, as non-threatening by sheep (Kendrick & Baldwin, 1987).

However, this was not the same for the response of the sheep in relation to the dog barking tape when compared to that of the dog. There was a significant difference between the behaviour of the sheep in the presence of the dog and dog barking tape, (Figure 4.3). This suggests that the sheep did not find the dog barking tape as aversive as the dog and the results suggest that the sheep viewed the dog barking tape to be the least aversive stimulus shown – less aversive than the goat and the sound of a drum beat. This contrasts with previous studies which have found that the presence of a dog or a recording of a dog barking causes elevations in plasma cortisol, ACTH, heart rate and body temperature above those observed upon exposure to humans and noise (Harlow et al., 1987; Baldock & Sibley, 1990; Cook, 1996; Komesaroff et al., 1998, cited in Dwyer & Bornett, 2004). This result suggests that sheep either regard auditory predatory stimuli in a different context to that of visual predators, or more plausibly, that the recording of a dog barking used in this study, simply did not accurately represent a real or live threat to the animals due to poor recording or sound quality.

The behavioural responses of the sheep in this study may have been affected by the amount of experience each of the sheep may have had with any one of the four stimuli, as suggested by Cockram (2004). However, each of these test animals had been born and run together on the same farm and their experiences, therefore, are expected to be as consistent as any group of normal farm animals. These sheep were raised outdoors, with some regular contact with humans and dogs. Most of this contact would be considered aversive, e.g. herding, yarding, docking, and drenching (Beausoleil et al., 2005). However, this contact represents the normal experience of extensively managed sheep in New Zealand, the behavioural responses of which we wished to study in the presence of a predator. Because experience with a specific dog, or goat will influence a sheep's subsequent behavioural response to that stimulus, all stimuli used were unfamiliar to the test sheep.

The difference in age between the sheep used in this experiment and the lambs used in chapter two appeared to have a large impact on the behaviour of the animals, and the results of this study. With age comes experience, and it is this experience that appears to have determined whether the animals responded to the stimuli in relation to the potential threat posed. Dwyer (2004) suggested that the way sheep view potential predators appears not to be innate, but is instead dependent on the previous experience of the sheep. The evidence from this study, especially when compared with the study in Chapter 3, supports this notion.

Therefore the results from this study found that sheep find dogs aversive, and will withstand significantly more pressure in their presence than with a goat. This suggests that sheep are able to alter their behaviour in the presence of a potential threat. Furthermore, this study supports the idea that the way that sheep view potential predators is not innate, but learned from previous experience.

5.0 General Discussion

Prey species are said to be under selection pressure to hide any signal that a predator might use to detect compromised escape ability, and thus an injured animal may conceal any behaviour that signals it is in pain (Broom, 2001). Given the difficulties associated with studying predator prey interactions in the wild, especially those associated with assessing the health of captured prey before they are consumed by their captor, this thesis involved two controlled experiments using treatments that are known to cause pain in sheep. The response of lambs and ewes to these treatments, were tested in the presence of various stimuli.

In Study 1 (Chapter 3), 384 lambs aged between 1-6 weeks of age were subjected to routine castration or left intact. Castration is known to be a painful husbandry procedure. Lambs were placed in a pen following either of two treatments (castration or control handling) with one of four stimuli present (dog, goat, box or bark). In addition, half of these lambs (N=192) were tested under the same conditions, with their dams present. If the assumptions of Broom (2001) are correct, we hypothesised that lambs should show fewer behaviours indicative of the pain caused by castration in the presence of aversive stimuli such as a predator.

We hypothesised that the behaviour of castrated lambs would differ to that of control lambs, and that castrated lambs would show less castration pain behaviour in the presence of a predator (dog and dog barking tape), than when a biologically insignificant novel object (cardboard box) or a goat were present. Finally it was hypothesized that lambs would behave differently in the presence of their dam, in the presence of a predator because the dam would be more experienced, and hence cue her lamb in some way of the potential danger.

In Study 2 (Chapter 4), 16 mixed aged ewes were subjected to a mechanical pressure device attached to their back left leg. This is known to elicit a pain reflex response, characterised by the lifting of the affected back leg of the sheep once a certain threshold is reached. Individual sheep were placed in a metabolic crate, and a minimum of four-baseline threshold results were recorded, until four consistent results were obtained. One of four stimuli (dog, goat, drum beat, bark) was then placed in an adjacent pen, and another threshold result was recorded.

If the assumptions of Broom (2001) are correct, we hypothesised that sheep should increase their pressure thresholds in the presence of aversive stimuli such as a predator, in an attempt to reduce their chances of being taken by a predator. We hypothesised that sheep would increase their pressure thresholds significantly more in the presence of a predator (dog and bark), then when an arbitrary noise (drum beat) was played, or a goat was present.

As hypothesised, castrated lambs exhibited more restlessness behaviour than non-castrated flock mates. However, in contrast to our hypothesis, when lambs were castrated, and placed in the presence of a predator, their restlessness behaviour was similar to that of lambs that were castrated, but not exposed to a predator, but exposed to novel stimuli such as a box or a goat. Lambs also exhibited less restlessness behaviour when their dam was present in comparison to when their dam was absent, which supports the hypothesis that lambs would behave differently in their dams' presence.

Furthermore, adult sheep significantly increased their pressure thresholds in the presence of the dog stimulus in comparison to when the goat was present. However, in contrast to our hypothesis, there was a significant difference between the behaviour of the sheep in the presence of the dog and dog barking tape, (Figure 4.3). This suggests that the sheep did not find the dog barking tape as aversive as the dog, and the results suggest that the sheep viewed the dog barking tape to be the least aversive stimulus shown – less aversive than the goat and the sound of a drum beat.

In Chapter 3 of this thesis, lambs were used as the test subjects because their naivety meant that they had had no experience with any of the four stimuli (box, dog, goat, bark). However, due to this naivety, lambs appeared not to find any of the stimuli more aversive than the next. It is likely that the lambs were reacting to the novelty of both the situation and the stimuli, as they had not yet learned to make negative associations with any of the four stimuli specifically. Porter & Bouissou (1999) found that lambs did not respond with aversion to images of dogs as adult ewes did, suggesting that the lambs had not yet had enough experience with dogs to link unpleasant associations with these images.

In comparison, the adult sheep (Chapter 4), had had previous experience with dogs and may have had previous experience with the other 3 stimuli. They may have formed negative associations with some of these stimuli (Dwyer, 2004), and their behavioural responses to the stimuli, as well as being statistically significant, support anecdotal accounts that prey animals will behave differently in the presence of a predator (Kruuk, 1972; Schaller, 1972; Bateson, 1991).

It is possible that the methods used in this thesis (especially in Chapter 3), were not sensitive enough to detect altered prey behaviour in the presence of a predator. In future trials, it may be better to measure a larger number of behavioural indices. For example, only restlessness behaviour was examined in the study of castrated lambs. However, other behaviours have been used to measure castration associated pain behaviour, including abnormal walking, tail wagging, and lip curling (Molony & Kent, 1997; Dinniss et al., 1999; Thornton & Waterman-Pearson, 1999). By measuring other behaviour indices in conjunction with restlessness behaviour, a more accurate picture may be been formed about an animal's pain response in the presence of each of the four stimuli.

Additionally, the movement of lambs in relation to the position of the stimulus may show how threatening the lambs view the stimulus to be. It has been shown that sheep will maintain a close distance to a cardboard box, whilst maintaining a larger distance from a dog when given the choice (Beausoleil et al., 2005). In this study, I saw similar behaviour in the lambs, although I did not quantify it. The lambs appeared to move closer to the cardboard box than to the other stimuli. Beausoleil et al. (2005) used an arena test to create approach/avoidance motivational conflict to detect differences in stimulus aversiveness. Testing the movement of lambs in relation to the stimulus, and incorporating a motivational conflict would test the animals desire to escape the presence of aversive, and therefore show how aversive the animal views the stimuli to be. This behaviour should be measured in future work. Moreover, future work should also measure physiological indices at the same time as measuring behaviour. This would add extra value to the data obtained, as a means of validation.

Using sheep, a highly domesticated animal as the test species in this thesis may have been problematic. Removed from natural predators, and selectively bred for their temperament, domestic sheep may no longer behave as wild sheep do in response to predation in their natural habitat. Nevertheless, domestic sheep appear to have retained many behavioural characteristics of wild sheep including their use of habitat, and in their social organization, reproductive and other behaviours (Hafez & Scott, 1962; Lynch & Alexander, 1973; Shackleton & Shank, 1984, cited in Dwyer, 2004). Physiological data suggest that thresholds for eliciting stress responses and behaviours associated with fear, may be elevated in domestic sheep (Hemmer, 1990; Kunzl & Sachser, 1999, cited in Dwyer, 2004). However, there is no evidence to suggest that these behaviours are not expressed once that threshold has been reached. Fear and anxiety-related behaviours have adaptive value in promoting survival in domestic and wild sheep (Boissy, 1998, cited in Dwyer, 2004), and are therefore considered components of the antipredator response (Frid & Dill, 2002). Thus, the antipredator strategies that evolved in wild sheep should persist in domestic animals even in the absence of natural predators (Byers, 1997, cited in Dwyer, 2004).

In future, work such as this should include the use of a control of “no stimulus”, and the animals should be run through the test pen a few times prior to the start of the experiment (as was done in Chapter 4). This would remove the effects of the novelty of the environment, making it easier to distinguish whether the lambs are reacting to the novelty of the situation, or whether they are reacting to the presence of the stimuli. Because there is no significant difference amongst the results of this study between predator and novel stimuli it is hard to conclude whether the test animals were hiding their pain, or withstanding more pain as a consequence of reacting to the stimuli, or whether they were reacting to the novelty of the situation. If there was a "no stimulus" control, and the sheep and lambs showed more pain behaviour when there were no stimuli present, then it would show they viewed all of the four stimuli as dangerous, and were therefore capable of hiding their pain.

One of the most interesting results from this study is the effect the lamb's dam has on her lambs' behaviour. Future studies could further investigate this relationship, and find out whether the mother was actually cueing her young, and, if so, what cues she was using to do this. Juvenile prey animals are vulnerable to a wider range of predators than adults due to their small size (FitzGibbon, 1990a), so it would be advantageous for them to closely follow the actions of their mother in dangerous situations. Furthermore, because dams have a greater wealth of experience with predators than lambs, it is assumed that mothers that give cues to lambs, and their lambs respond to these cues, are more likely to have surviving offspring. It is therefore in the lamb's best interest to rely on cues from their mother in regards to dangerous situations.

How prey animals view humans is important in relation to pain assessment and future work should specifically focus on this. In a veterinary setting, if prey animals view humans as predators, they may hide their pain from humans, making assessment difficult. In this context, whether sheep are more fearful of humans or dogs is not known and the nature of the relationship between humans and sheep is uncertain (Rushen, 1990). It is possible humans could be viewed as dominant conspecifics, or as predators (Beausoleil et al., 2005). However, neurophysiological evidence suggests that dogs and humans are recognized with similar emotional significance (Kendrick & Baldwin, 1987). When placed in an arena test, Beausoleil et al. (2005) found that sheep regarded dogs as more aversive than humans. How sheep respond to humans when in pain is unknown, and could provide valuable information for farmers carrying out painful husbandry procedures on sheep, such as castration and tail docking. In this thesis, the presence of humans was a confounding variable that was not controlled for. In Chapter 3, humans were intermittently visible to the test lambs, and in Chapter 4, humans were standing behind the test sheep recording the pressure threshold results. However, the level of human presence remained consistent for all experiments, and in the presence of all four stimuli. Nevertheless, it is difficult to conclude whether the animals were responding to the human presence or that of the stimuli, or a combination of the two. Future work should remove this human presence variable by carrying out an experiment such as that in Chapter 3 in an enclosed room, where the lambs can be placed into a room and video-taped with no human presence.

In line with anecdotal evidence, the results from this thesis found that adult sheep find dogs aversive, and will withstand significantly more pressure in their presence than with a goat. This suggests that sheep are able to alter their behaviour based on the intensity of the threat. Furthermore, this study supports the idea that the way that sheep view potential predators is not innate, but learned from previous experience.

Because of their inability to self-report pain, overt behaviour is still the best method available to quantify pain in animals. The possibility that prey animals may hide their pain from predators poses problems for using behaviour as a pain assessment tool. However, some of the best research in this field has been able to encompass this impediment. Work carried out by Lester et al. (1991, 1996) and Dinniss et al. (1997, 1999) comparing the behaviour of controls to that of animals following surgery, with and without anaesthetics or analgesics has shown that if an analgesic can reduce certain behaviours exhibited by an animal following surgery to levels exhibited by controls, then this behaviour is occurring as a result of the surgery, and can be assumed to be pain related.

In future, more work following these principles needs to carried out to establish a unique set of behaviours for a wide range of species and injuries or surgical procedures as the pain following injuries or procedures can not only differ between species and individuals but they can also be injury specific. Two further avenues for future research include determining how prey animals view humans, and further investigating the mother-young relationship, and the affect the presence of a predator has on this.

6.0 References

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7.0 Appendices

7.1 Appendix A – Castration Experiment Data

7.1.1 Castration Restlessness Data

Results from each of the four castrated lambs per pen.

Treatment	Day	Stimulus	Pen	Lamb 1	Lamb 2	Lamb 3	Lamb 4
Castrated	1	BARK	A	40	18	52	23
Castrated	1	BARK	B	57	43	31	9
Castrated	1	BARK	B	48	35	55	72
Castrated	1	BOX	A	27	21	9	30
Castrated	1	BOX	A	80	26	61	71
Castrated	1	BOX	B	15	14	15	18
Castrated	1	DOG	A	22	7	16	9
Castrated	1	DOG	B	51	41	17	33
Castrated	1	DOG	B	17	27	25	2
Castrated	1	GOAT	A	7	25	22	32
Castrated	1	GOAT	A	91	71	29	24
Castrated	1	GOAT	B	19	17	10	41
Castrated	2	BARK	A	44	11	16	44
Castrated	2	BARK	B	40	39	39	16
Castrated	2	BARK	B	27	36	58	23
Castrated	2	BOX	A	26	49	29	25
Castrated	2	BOX	A	38	57	2	49
Castrated	2	BOX	B	16	63	75	53
Castrated	2	DOG	A	81	54	25	37
Castrated	2	DOG	A	23	16	28	64
Castrated	2	DOG	B	15	6	18	16
Castrated	2	GOAT	A	25	39	21	43
Castrated	2	GOAT	B	43	27	5	55
Castrated	2	GOAT	B	33	21	27	45
Castrated	3	BARK	A	19	7	21	35
Castrated	3	BARK	A	43	2	14	19
Castrated	3	BARK	B	15	31	.	.
Castrated	3	BOX	A	7	19	29	.
Castrated	3	BOX	A	15	56	.	.
Castrated	3	BOX	B	17	43	32	45
Castrated	3	DOG	A	7	.	.	.
Castrated	3	DOG	B	51	5	4	31
Castrated	3	DOG	B	32	17	32	29
Castrated	3	GOAT	A	15	0	25	35
Castrated	3	GOAT	A	30	5	13	21
Castrated	3	GOAT	B	3	1	10	11
Castrated	4	BARK	A	0	4	13	59
Castrated	4	BARK	A	8	15	12	25
Castrated	4	BARK	B	9	51	11	0
Castrated	4	BOX	B	14	35	23	30
Castrated	4	BOX	B	4	13	2	.

Castrated	4	BOX	B	38	47	31	9
Castrated	4	DOG	A	10	23	6	17
Castrated	4	DOG	A	52	33	38	4
Castrated	4	DOG	B	4	4	22	17
Castrated	4	GOAT	A	0	20	33	22
Castrated	4	GOAT	B	21	15	13	43
Castrated	4	GOAT	B	15	19	2	23

7.1.2 Control Restlessness data

The results from each of the four control lambs per pen.

Treatment	Day	Stimulus	Pen	Lamb 1	Lamb 2	Lamb 3	Lamb 4
CONTROL	1	GOAT	A	3	9	7	5
CONTROL	1	BOX	A	4	4	3	0
CONTROL	1	DOG	A	1	2	0	2
CONTROL	1	GOAT	A	2	0	0	6
CONTROL	1	BOX	A	0	0	0	13
CONTROL	1	DOG	B	10	1	0	3
CONTROL	1	GOAT	B	0	1	1	2
CONTROL	1	BOX	B	0	1	2	0
CONTROL	1	DOG	B	0	0	0	0
CONTROL	1	BARK	B	9	3	0	0
CONTROL	1	BARK	B	0	2	0	0
CONTROL	1	BARK	A	0	0	2	5
CONTROL	1	BARK	A	0	0	0	0
CONTROL	1	BARK	B	7	0	0	0
CONTROL	1	BARK	B	6	0	0	6
CONTROL	1	DOG	A	2	5	2	3
CONTROL	1	BOX	A	0	0	0	4
CONTROL	1	GOAT	A	0	4	0	0
CONTROL	1	BOX	A	0	0	0	0
CONTROL	1	DOG	A	0	0	0	0
CONTROL	1	GOAT	B	1	0	3	1
CONTROL	1	DOG	B	0	0	0	0
CONTROL	1	BOX	B	0	0	11	3
CONTROL	1	GOAT	B	2	3	4	7
CONTROL	2	DOG	B	0	0	0	0
CONTROL	2	BOX	B	0	6	2	0
CONTROL	2	GOAT	B	0	2	5	2
CONTROL	2	DOG	B	1	0	0	0
CONTROL	2	BARK	A	0	8	0	0
CONTROL	2	BARK	A	0	0	0	0
CONTROL	2	BARK	B	6	0	0	0
CONTROL	2	GOAT	A	0	3	0	0
CONTROL	2	BOX	A	0	5	0	2
CONTROL	2	DOG	A	0	0	0	0
CONTROL	2	GOAT	A	0	0	1	0
CONTROL	2	BOX	A	0	0	0	0
CONTROL	2	DOG	A	0	0	0	0
CONTROL	2	GOAT	A	0	0	0	0
CONTROL	2	DOG	A	5	2	0	0
CONTROL	2	BARK	A	0	0	0	0
CONTROL	2	BARK	A	0	0	0	0

CONTROL	2	BOX	B	0	0	0	0
CONTROL	2	GOAT	B	0	0	0	0
CONTROL	2	BOX	B	0	0	0	0
CONTROL	2	DOG	B	0	0	0	0
CONTROL	2	GOAT	B	0	0	0	0
CONTROL	2	BOX	B	0	3	0	0
CONTROL	2	BARK	B	0	0	2	0

7.1.3 Time First Sat Data

Mother Present/Absent	Stimulus	Lamb 1	Lamb 2	Lamb 3	Lamb 4
Absent	Goat	18.31	13.6	5.85	5.7
Absent	Box	13.98	13.04	15.63	10.56
Absent	Dog	8.23	21.65	20.56	20.37
Absent	Box	8.1	5.27	5.17	14.15
Absent	Goat	4.27	5.18	7.17	11.2
Absent	Dog	2.43	7.77	13.47	1.93
Absent	Goat	14.25	12.06	22.52	0.19
Absent	Box	21.37	12.58	8.46	13.48
Absent	Dog	15.86	6.1	7.92	24.68
Absent	Dog	8.56	4.01	2.49	13.64
Absent	Box	9.69	1.44	14.74	3.16
Absent	Goat	12	11.53	17.42	4.43
Absent	Box	10.04	10.88	24.55	2.18
Absent	Dog	12.82	19.18	15.38	0.55
Absent	Bark	0.47	12.34	2.47	2.08
Absent	Goat	5.34	4.42	21.41	1.19
Absent	Dog	13.23	19.77	14.57	16.92
Absent	Box	12.53	1.49	0.5	5.57
Absent	Goat	7.96	6.29	0.54	4.46
Present	Dog	15.43	.	.	.
Present	Goat	14.15	.	6.69	2.11
Present	Goat	7.21	22.53	20.06	4.06
Present	Box	20.45	19.16	14.67	.
Present	Bark	13.92	26.45	18.84	16.43
Present	Box	18.23	0.8	.	.
Present	Dog	12.56	23.32	23.67	3.41
Present	Box	16.19	8.42	2.48	8.22
Present	Dog	7.85	20.45	1.3	4.8
Present	Dog	15.26	8.58	21.88	16.58
Present	Dog	6.04	12.67	12.3	24.91
Present	Bark	.	21.17	15.6	5.61
Present	Bark	16.97	18.76	20.12	13.48
Present	Goat	.	20.12	12.56	14.91
Present	Goat	17.88	16.99	18.81	12.23
Present	Box	10.84	4.5	13.43	6.9
Present	Dog	21.66	22.99	16.38	15.65
Present	Goat	17.99	19.31	23.19	7.57
Present	Box	22.94	15.71	25.58	.
Present	Bark	19.11	9.58	17.29	.

7.1.4 Average Time Spent Sitting

Mother Present/Absent	Stimulus	Pen	Result
Absent	G	A	0.990943
Absent	G	A	0.672166
Absent	G	B	0.841697
Absent	G	A	0.595472
Absent	G	B	0.728695
Absent	G	B	0.832445
Present	G	A	0.642005
Present	G	A	0.609203
Present	G	B	0.754135
Present	G	A	0.343916
Present	G	B	0.570112
Present	G	B	0.499933
Absent	D	A	0.863683
Absent	D	B	0.723112
Absent	D	B	0.790074
Absent	D	A	0.655971
Absent	D	A	0.487859
Absent	D	B	0.635027
Present	D	A	.
Present	D	B	0.722167
Present	D	B	0.692896
Present	D	A	0.620184
Present	D	A	0.555319
Present	D	B	0.5959
Absent	BK	A	0.648735
Absent	BK	B	0.63193
Absent	BK	B	0.497328
Absent	BK	A	0.572704
Absent	BK	B	0.52052
Absent	BK	B	0.734752
Present	BK	A	0.898543
Present	BK	A	0.514532
Present	BK	B	.
Present	BK	A	0.473561
Present	BK	A	0.504251
Present	BK	B	0.467011
Absent	BX	A	0.779555
Absent	BX	A	0.483253
Absent	BX	B	0.849522
Absent	BX	A	0.642873
Absent	BX	A	0.444596
Absent	BX	B	0.566533
Present	BX	A	.
Present	BX	A	.
Present	BX	B	0.596393
Present	BX	B	0.675306
Present	BX	B	0.829606
Present	BX	B	.

7.2 Appendix B – Pressure Threshold Experiment Data

7.2.1 Baseline and Stimulus pressure threshold readings in the presence of the dog stimulus

Average Baseline values calculated from four consecutive readings, and the stimulus pressure threshold value.

	Dog		
Sheep No.	Average Baseline	Stimulus Value	Day
1	10.2	9.2	1
2	7.45	8.8	1
5	9.275	11.6	1
12	11.5	13	1
15	4.375	4	1
13	2.125	4	2
5	7	7	2
9	5.875	12	2
12	7.625	10	3
16	2.75	4	3
4	7.375	4.5	3
8	5.375	10	3
11	9.5	20	4
15	2.375	6	4
3	2.125	2	4
7	5	6.5	4

7.2.2 Baseline and Stimulus pressure threshold readings in the presence of the noise stimulus

Average Baseline values calculated from four consecutive readings, and the stimulus pressure threshold value.

	Noise		
Sheep No.	Average Baseline	Stimulus Value	Day
4	8.675	10	1
6	3.7	6.7	1
11	7.975	8	1
13	3.275	3	1
10	7.125	7	2
14	5.875	5	2
2	7.375	7	2
6	5.875	5	2
15	3.25	3	3
3	5.125	3	3
7	7.75	14	3
11	7.5	8	3
5	5	5	4
9	6.125	7	4
13	2	2	4
1	6.375	7.5	4

7.2.3 Baseline and Stimulus pressure threshold readings in the presence of the goat stimulus

Average Baseline values calculated from four consecutive readings, and the stimulus pressure threshold value.

	Goat		
Sheep No.	Average Baseline	Stimulus Value	Day
7	7.95	10.6	1
10	9.325	9	1
16	2.175	3.5	1
7	6	11	2
11	8.125	8	2
1	9.75	9.5	2
15	2	2	2
3	4	2.5	2
6	3.875	3.5	3
10	8.75	7	3
14	7	10	3
2	3.75	3.5	3
16	3	3	4
4	8.5	4.5	4
8	6.875	7.5	4
12	7.625	8	4

7.2.4 Baseline and Stimulus pressure threshold readings in the presence of the bark stimulus

Average Baseline values calculated from four consecutive readings, and the stimulus pressure threshold value.

	Bark		
Sheep No.	Average Baseline	Stimulus Value	Day
3	4.925	6.2	1
8	7.75	10.5	1
9	5.925	7	1
14	7.5	4	1
8	8.625	7	2
12	13.25	5	2
16	3.625	3.5	2
4	8.625	4.5	2
9	10	8	3
13	3	3	3
1	8	16	3
5	5.5	6.5	3
14	4.875	5	4
2	5.75	2.5	4
6	5.5	3	4
10	5.375	3.5	4

7.3 Appendix C – ANIMAL ETHICS APPLICATIONS

AEC/5 (Amended 10/00)

Animal Ethics Committee

To: Secretary
Animal Ethics Committee
Old Main Building
Turitea, Palmerston North

Please send this original (1) application plus thirteen (13) copies
Application due one week prior to the meeting

APPLICATION FOR APPROVAL OF PROPOSED EXPERIMENTAL/TEACHING PROCEDURES USING LIVE ANIMALS

1. CHIEF APPLICANT: *(staff member only)*

(a) **Name** Edward O. Minot
Qualifications BSc MSc DPhil
Position Associate Professor
Inst/Sch/Dept INR

2. OTHER APPLICANTS: *(see Code Section 5.1 for those who should be listed)*

(a) **Name** Kevin J. Stafford
Qualifications MVB PhD
Position Associate Professor

(b) **Name** Suzanne Thomas
Qualifications BSc
Position MSc student

(c) **Name**
Qualifications
Position

OFFICE USE ONLY

Date Received: RECEIVED 26 SEP 2003 Equity & Ethics

Copy for:
Applicant
Head of Institute/Department
Office ✓

Date sent: 10/10/03

Protocol No: 03/111

Decision: Appr.

MASSEY UNIVERSITY ANIMAL ETHICS COMMITTEE APPROVED

3. **DETAILS OF PROJECT:**

(a) **Title** Does the presence of a predator influence lamb behaviour after castration

(b) **Commercial sensitivity status** **Yes** [ ]

No [X]

4. **JUSTIFICATION OF PROJECT:**

(a) **What are the expected benefits of the proposed work and how will the new knowledge be communicated to others?** *(Benefits may include improved basic knowledge, improved animal health, teaching)*

In the veterinary and ecology literature it is frequently stated that prey species, when injured or diseased, will attempt to behave in a normal manner i.e. similar to conspecifics, so as not to draw the attention of a predator. This hypothesis has not been tested. It is a significant hypothesis as the lack of overt behaviour indicative of pain in some farm animals after painful husbandry procedures such as castration has been interpreted to indicate that the animal is not experiencing pain. It may be that the human observer is perceived as a predator and affects the animal's behaviour.

Lambs are restless after ring castration, standing up and lying down many times in the 30 minutes following the procedure. If the presence of a dog, which is generally considered a significant predator for sheep, has no effect on lamb behaviour after castration then it is probable that the lamb has a predetermined pattern of behaviour that is not influenced by environmental factors or the lamb does not identify the dog as a predator. The lamb ewe dyad is used in this trial to eliminate the latter possibility. If the presence of a predator influences the pattern of behaviour then behaviour as an index of pain in livestock can only be used within a defined environment.

Thus the effect of a predator on animal behaviour may have significant implications on our use of behaviour to identify whether animals are or are not experiencing pain.

(b) **Why is it necessary to use animals for this activity?** *(The term "animal" is defined in the Code of Ethical Conduct, Item 9)*

This study investigates the behaviour of sheep and cannot be done in any other way.

5. **DESCRIPTION OF PROCEDURES AND MANIPULATIONS:** *(Manipulation is defined in the Code of Ethical Conduct, Item 9)*

(a) **Give a brief description of your trial design/teaching demonstration.** *(One or two paragraphs)*

384 lambs will be used in this trial and exposed for the 30 minutes after castration to one of four different stimuli either in a group of lambs only or in the company of their mothers. Half of the lambs will be castrated during the trial.

Groups of 64 to 80 ram lambs and their ewes will be brought into the yards of the wool shed at Tuapaka on each morning of the trial. The lambs will be used in groups of 8 at a time. Immediately before a trial the lambs and ewes will be separated. The experimental group of lambs (4 lambs) will be castrated by rubber ring and the control group (4 lambs) will be caught and their scrotum handled but will not be castrated. There will be two lamb treatments; **Lambs alone** – lambs will be separated from their mothers until the end of the trial, and **Lambs with ewes** – lambs and ewes are placed in a pen but held separate by a gate through out the trial.

The observation pen is approximately 3x3m and will hold 8 lambs at a time for each trial, 4 castrated and 4 controls. The 8 ewes will be held in two adjacent pens and will be able to contact their lambs through a gate. Each lamb will be identified with a number spray painted on its back. Only one ram lamb from a set of ram lamb twins will be used. The stimuli will be presented in random order throughout the day.

During a trial, one of four stimuli will be presented to the lambs for 30 minutes and the lamb behaviour will be videotaped for this period of time. The stimuli, a cardboard box, a dog, a goat and a tape recorder playing the noise of a dog barking will be placed at one end of the pen. Between 64 and 80 lambs will be used each day and the trial will take up to 6 days. The behaviour of all lambs will be recorded and any differences between the castrated and control lambs subjected to the stimuli will be analysed using MANOVAs.

At the end of each day all the control lambs will be castrated and all lambs will be tailed before being released back onto pasture with their mothers.

Protocol

	Lamb Treatment			
	Lambs alone		**Lambs with ewes**	
Treatment	Castrated	Control	Castrated	Control
Dog	24 B	24 A	24 B	24 A
Barking	24 B	24 A	24 B	24 A
Goat	24 B	24 A	24 B	24 O
Box	24 B	24 A	24 B	24 O

O=No suffering A=Little suffering B=Moderate suffering

(b) **How many animals will you use and how have you determined the number of animals to be used? Where a power analysis is appropriate, provide details to justify animal numbers.**

384 lambs in 16 groups of 24. Previous research suggests that the use of 4 animals in each treatment group with 6 replications of each treatment will allow the statistical tests to have sufficient power to detect meaningful differences in behaviour. 192 ewes will be present for 8 of the 16 groups and a further 192 ewes will be separated from their lambs for the duration of the 30-minute trials.

(c) **Describe the manipulations to be performed on the animals.**

These lambs will be subjected to castration and tail docking using rubber rings as part of normal farm practice. The application is for permission to observe the lambs after castration with or without their dams and subject to exposure to specified stimuli. 384 lambs and about 384 ewes will be involved.

(d) **How will the proposed manipulation affect the well-being of the animals?**

Castration is painful and stressful and separating the lamb from their dam is also moderately stressful for lambs and ewes (384). The presence of the dog and the barking may be stressful but the goat and box will not be. The presence of the dam might reduce the stress caused by the dog and barking.

(e) **Describe any restraint applied to the animals.**

Lambs will be held in a small pen with their dams and then held in an observation pen for 30 minutes. Lambs will be caught and held to allow castration and tailing. All lambs will be caught three times to allow spray painting, treatment (castration, control handling) and after the trial tailing with or without castration. Ewes will be held in a wool shed and half of them will be caught and moved into pens beside their lambs.

6. CARE OF ANIMALS:

(a) **What access will the animals have to water?**

Water will be available at all times except in the holding pens and observation pens.

(b) **Describe the feeding regimen for the animals.**

Food will be withheld during the trial from ewes. Lambs will be held separate from their dams for less than an hour.

(c) **From where will the animals be obtained?**

Massey Farms

(d) **Where will the animals be kept throughout the study period?**

In the yards and wool shed at Tuapaka Farm, Massey University

(e) **Who is responsible for the routine care and health surveillance of the animals?**

The farm manager

(f) **If the Chief Applicant is unavailable, who will make decisions if emergency care is required?**

Kevin Stafford

7. FATE OF ANIMALS:

(a) **What will happen to the animals at the completion of the study?**

They will be returned to their paddocks

(b) **If any animals are to be euthanased, describe the method.**

NA

8. ALLEVIATION OF IMPACT OF MANIPULATIONS:

(a) **What features of the manipulations minimise their impact on the animals?**

In several previous trials we have observed lambs after castration without any obvious problems.

(b) **Stipulate the use (and dose rate and route of administration) of any anaesthesia, analgesia, sedative, tranquilliser or other pharmacological agent applied to reduce the impact of manipulations on the animals.**

None will be used

(c) **What frequency of monitoring is to be maintained?**

Continuous monitoring during the trial itself.

(d) **What advice regarding identification of any expected adverse effects will be given to staff responsible for the ongoing care of the animals?**

There should be no problems.

9. **EXPERIENCE OF APPLICANTS:**

(a) **What is the experience of the applicants with the techniques being used in this project?**

Kevin Stafford (10 years) has carried out many trials similar to this one on lambs and Suzanne Thomas comes off a sheep farm. .

(b) **If an applicant is using a technique with which he/she has no previous experience, what training will be provided?**

NA

(c) **List the people providing professional services and the services provided.** *(These personnel need not be applicants - see Code of Ethical Conduct, Item 5.1)*

10. **SIGNATURES OF APPLICANTS:**

I certify that:

(a) I have read the Massey University Code of Ethical Conduct for the Use of Live Animals for Teaching and Research and agree to comply with its requirements throughout the duration of the proposed procedures;

(b) to the best of my knowledge, this protocol or one substantially like it has not been declined by another Animal Ethics Committee.

Note: Carefully read (a) and (b) above before signing

Signature(s) of Applicant(s)	Printed Name(s) of Applicant(s)
[signature]	Edward O. Minot
[signature]	Kevin Stafford
[signature]	Suzanne Thomas

Date: 24/09/03

11. APPROVED BY DESIGNATED SIGNING AUTHORITY:

I have read this application and agree that it meets the intent and spirit of the Massey University Code of Ethical Conduct for the Use of Live Animals for Teaching and Research:

Signed: [signature] Date: 25/4/03

Name: B P. Springett, Institute of Zoology.

Institute: INR.

12. MAF STATISTICS FORM:

Please ensure that an "Animal Use Statistics" form is completed and attached.

NOTES:

(a) The staff member with signing authority delegated from MUAEC must not sign his/her own application in Section 10 above. Please obtain the signature of another staff member with delegated authority.

(b) Any departure from an approved protocol that adversely affects the welfare or increases the number of animals must be approved by the Chair of MUAEC acting with authority vested through paragraph 5.10 of the Code of Ethical Conduct. However, in the case of modifications of a minor nature only, these may be approved in writing by any staff member with delegated signing authority. A description of such minor modifications (including approval) shall be submitted to the Secretary of MUAEC who will attach it to the original protocol and note it on the agenda for the next meeting. Further copies shall be attached to the protocols held by the Institute and the Chief Applicant (Code 5.6).

Protocol ID
03/111

ANIMAL USE STATISTICS
APPLICATION/FINAL RETURN FORM

(Amended 11/2/01)

If more than one animal type is required then fill in one form for each type.

Application: When applying to the AEC for approval of a manipulation the applicant should complete Box 1 and enter in boxes 2 to 7, in the *'Planned'* column (P), the appropriate figures for the number of animals required.

Final return: When the manipulation is completed, Boxes 2 to 10 should then be completed in the *'Used'* column (U) by entering appropriate figures for the number of animals which were actually used.

Chief Applicant: E O Minot

Inst/Sch/Dept: INR

Title of Project: Does the presence of a predator influence lamb behaviour after castration

1. Animal type: Sheep Lambs and ewes Code: 1f

(see bottom of this form)

2. Source of animals (number)

		P	U
Breeding unit	A		
Commercial	B		
Farm	C	768	
Born during project	D		
Captured	E		
Imported	F		
Public sources	G		
TOTAL = A			

3. Status of animals (number)

		P	U
Normal/conventional	a	768	
*SPF/germ free	b		
Diseased	c		
Transgenic/chimaera	d		
Protected species	e		
Unborn/prehatched	f		
Other	g		

* *Specific pathogen free*

4. Main category of manipulation/use (enter the total from 2 above in one box only)

		P	U			P	U			P	U
Teaching	A			Animal husbandry	d			Veterinary research	g		
Species conservation	B			Basic biological research	e	768		Commercial	h		
Environmental management	C			Medical research	f			Other	j		

5. Any re-use of animals (number to be inserted)

		P	U			P	U	
No prior use	a	768		Previously used	b	0		Total a + b =

AEC/5 (Amended 10/00)

Animal Ethics Committee

To: Secretary
Animal Ethics Committee
Old Main Building
Turitea, Palmerston North

Please send this original (1) application plus thirteen (13) copies
Application due one week prior to the meeting

APPLICATION FOR APPROVAL OF PROPOSED EXPERIMENTAL/TEACHING PROCEDURES USING LIVE ANIMALS

1. **CHIEF APPLICANT:** *(staff member only)*
   (a) **Name** Ed Minot
   Qualifications PhD
   Position Associate Professor
   Inst/Sch/Dept INR

2. **OTHER APPLICANTS:** *(see Code Section 5.1 for those who should be listed)*
   (a) **Name** Kevin Stafford
   Qualifications MVB PhD
   Position Professor

   (b) **Name** Suzanne Thomas
   Qualifications BSc
   Position MSc student

   (c) **Name**
   Qualifications
   Position

OFFICE USE ONLY

Date Received: RECEIVED 28 OCT 2003 Equity & Ethics

Copy for: Date sent: 17/11/03

Applicant

Head of Institute/Department

Office ✓

Protocol No: 03/122

Decision: F app.

MASSEY UNIVERSITY ANIMAL ETHICS COMMITTEE APPROVED

Date: 7/11/03

3. **DETAILS OF PROJECT:**

(a) **Title** Does the presence of a predator influence sheep pain threshold measurements

(b) **Commercial sensitivity status** Yes [ ] No [X]

4. **JUSTIFICATION OF PROJECT:**

(a) **What are the expected benefits of the proposed work and how will the new knowledge be communicated to others?** *(Benefits may include improved basic knowledge, improved animal health, teaching)*

In the veterinary and ecology literature it is frequently stated that an animal of a prey species, when injured or diseased, will attempt to behave in a normal manner, i.e. similar to healthy conspecifics, so as not to draw the attention of a predator. This hypothesis has not been tested. It is a significant hypothesis because the absence of overt behaviour indicative of pain in farmed animals after painful husbandry procedures has been interpreted to indicate that the animal is not experiencing pain. This study is one of a series to investigate the impact of predator presence on the behavioural expression of pain in sheep.

In this study, the pain threshold of sheep will be monitored under different conditions. The presence of a dog on the pain threshold of sheep will be measured. If the presence of a predator influences this, then behaviour as an index of pain in livestock can only be used within specific environmental contexts.

Whether or not the presence of a predator has an effect on the pain threshold and behaviour of sheep, the results have wider implications regarding our ability to identify when animals are experiencing pain.

(b) **Why is it necessary to use animals for this activity?** *(The term "animal" is defined in the Code of Ethical Conduct, Item 9)*

This study investigates the behaviour of sheep and cannot be done in any other way

5. **DESCRIPTION OF PROCEDURES AND MANIPULATIONS:** *(Manipulation is defined in the Code of Ethical Conduct, Item 9)*

(a) **Give a brief description of your trial design/teaching demonstration.** *(One or two paragraphs)*

This experiment looks at whether sheep, in the presence of a predator, will change the amount of pressure they will withstand before moving their leg. The following experiments will be carried out over a series of days in November - December 2003.

20 sheep will be used in this trial and exposed to four different stimuli. For each of the four stimuli a pain threshold value will be obtained from each sheep. The pain threshold technique is described in Ley et al. (1995; *Vet.Record* 137:85-87; attached to top copy).

Firstly, it needs to be established whether a sheep will consistently respond to the same level of pressure, in the absence of a predator. Each of the 20 Sheep will be trained to stand in a sheep box, and a pressure plate will be placed around the sheep's back, right leg. Pressure will be applied to the plate until the animal moves its leg. The amount of pressure applied until the sheep moves will be recorded for each test on each sheep, and will be referred to as the pain threshold. This method will be applied to each sheep until the sheep responds to the same pressure five consecutive times. This will be carried out for a maximum of twenty times, and then abandoned if five consecutive, consistent results are not obtained.

Once 5 consistent pain threshold values have been obtained for a particular sheep, this sheep will be subjected to one of the four stimuli, and a final threshold measurement will then be taken. This will then be repeated for each of the other sheep. Each sheep will be subjected to each of the four treatment stimuli over the course of the study. The four stimuli presented to the sheep will be a dog on a lead, a goat on a lead, the sound of a dog barking, and a novel sound such as loud machinery. Only one stimulus will be tested on each sheep per day. Approximately 16 sheep will be tested each day over a one week period. A residual effects Graeco-Latin square design will be used to randomise the treatments and times of day across the sheep. This design will maximise the information gained from the trial.

The sheep will be able to see five other 'buddy' sheep in surrounding boxes. There will be a total of 20 'buddy' sheep. This will mean that each buddy sheep will only spend a maximum of four hours in a box each day.

(b) **How many animals will you use and how have you determined the number of animals to be used? Where a power analysis is appropriate, provide details to justify animal numbers.**
20 Ewes will be used as test animals and 20 Ewes will be used as Buddy sheep so all together 40 Ewes will be required.

(c) **Describe the manipulations to be performed on the animals.**
The sheep will be trained to stand in a sheep box, and a pressure plate will be placed around their back right leg

(d) **How will the proposed manipulation affect the well-being of the animals?**
The pressure of the plate is a painful and moderately stressful procedure for all animals, but the sheep will be released as soon as they react to the pain.

(e) **Describe any restraint applied to the animals.**
The animal will be placed in a sheep box, which will be the only form of restraint, other than being held in a set of sheep yards.

6. CARE OF ANIMALS:

(a) **What access will the animals have to water?**
Water will be available at all times except in the pens and sheep box.

(b) **Describe the feeding regimen for the animals.**
Food will be withheld during the trial from ewes

(c) **From where will the animals be obtained?**
Massey University Veterinary Large Animal Teaching Unit Farm

(d) **Where will the animals be kept throughout the study period?**
At the Veterinary Large Animal Teaching Unit, Massey University

(e) **Who is responsible for the routine care and health surveillance of the animals?**
Kevin Stafford

(f) **If the Chief Applicant is unavailable, who will make decisions if emergency care is required?**
Ed Minot

7. FATE OF ANIMALS:

(a) **What will happen to the animals at the completion of the study?**
They will be returned to their paddocks

(b) **If any animals are to be euthanased, describe the method.**
NA

8. ALLEVIATION OF IMPACT OF MANIPULATIONS:

(a) **What features of the manipulations minimise their impact on the animals?**
This method has been carried out before with no problems

(b) **Stipulate the use (and dose rate and route of administration) of any anaesthesia, analgesia, sedative, tranquilliser or other pharmacological agent applied to reduce the impact of manipulations on the animals.**
None will be used

(c) **What frequency of monitoring is to be maintained?**
Continuous monitoring during the trial itself.

(d) **What advice regarding identification of any expected adverse effects will be given to staff responsible for the ongoing care of the animals?**
There should be no problems.

9. EXPERIENCE OF APPLICANTS:

(a) **What is the experience of the applicants with the techniques being used in this project?**
Kevin Stafford (10 years) has carried out many trials similar to this one.

(b) **If an applicant is using a technique with which he/she has no previous experience, what training will be provided?**
NA

(c) **List the people providing professional services and the services provided.** *(These personnel need not be applicants - see Code of Ethical Conduct, Item 5.1)*

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NA

(c) **List the people providing professional services and the services provided.** *(These personnel need not be applicants - see Code of Ethical Conduct, Item 5.1)*

10. SIGNATURES OF APPLICANTS:

I certify that:

(a) I have read the Massey University Code of Ethical Conduct for the Use of Live Animals for Teaching and Research and agree to comply with its requirements throughout the duration of the proposed procedures;

(b) to the best of my knowledge, this protocol or one substantially like it has not been declined by another Animal Ethics Committee.

Note: Carefully read (a) and (b) above before signing

Signature(s) of Applicant(s)	Printed Name(s) of Applicant(s)
[signature]	A/Prof Ed Minot
[signature]	Prof Kevin Stafford
[signature]	Suzanne Thomas

Date: 25 October 2003

11. APPROVED BY DESIGNATED SIGNING AUTHORITY:

I have read this application and agree that it meets the intent and spirit of the Massey University Code of Ethical Conduct for the Use of Live Animals for Teaching and Research:

Signed: [signature] Date: 28/10/03

Name: B.P. SPRINGETT

Institute: INR

12. MAF STATISTICS FORM:

Please ensure that an "Animal Use Statistics" form is completed and attached.

NOTES:

(a) The staff member with signing authority delegated from MUAEC must not sign his/her own application in Section 10 above. Please obtain the signature of another staff member with delegated authority.

(b) Any departure from an approved protocol that adversely affects the welfare or increases the number of animals must be approved by the Chair of MUAEC acting with authority vested through paragraph 5.10 of the Code of Ethical Conduct. However, in the case of modifications of a minor nature only, these may be approved in writing by any staff member with delegated signing authority. A description of such minor modifications (including approval) shall be submitted to the Secretary of MUAEC who will attach it to the original protocol and note it on the agenda for the next meeting. Further copies shall be attached to the protocols held by the Institute and the Chief Applicant (Code 5.6).

ANIMAL USE STATISTICS
APPLICATION/FINAL RETURN FORM
(Amended 11/2/01)

Protocol ID 03/1.22

If more than one animal type is required then fill in one form for each type.

Application: When applying to the AEC for approval of a manipulation the applicant should complete Box 1 and enter in boxes 2 to 7, in the *'Planned'* column (P), the appropriate figures for the number of animals required.

Final return: When the manipulation is completed, Boxes 2 to 10 should then be completed in the *'Used'* column (U) by entering appropriate figures for the number of animals which were actually used.

Chief Applicant: Edward Minot

Inst/Sch/Dept: Institute of Natural Resources

Title of Project: Sheep Pain Threshold in the Presence of a Predator

1. Animal type: Sheep Code: 1 f
(see bottom of this form)

2. Source of animals (number)

		P	U
Breeding unit	a		
Commercial	b		
Farm	c	40	
Born during project	d		
Captured	e		
Imported	f		
Public sources	g		
TOTAL = A			

3. Status of animals (number)

		P	U
Normal/conventional	a	40	
*SPF/germ free	b		
Diseased	c		
Transgenic/chimaera	d		
Protected species	e		
Unborn/prehatched	f		
Other	g		

* *Specific pathogen free*

4. Main category of manipulation/use (enter the total from 2 above in one box only)

		P	U			P	U			P	U
Teaching	a			Animal husbandry	d			Veterinary research	g		
Species conservation	b			Basic biological research	e	40		Commercial	h		
Environmental management	c			Medical research	f			Other	j		

5. Any re-use of animals (number to be inserted)

		P	U			P	U	
No prior use	a			Previously used	b	40		Total a + b = 40

6. Grading of manipulations (number in each grade to be inserted)

Description	Grade	P	U
A manipulation or use that causes no stress or pain or virtually no stress or pain. **No suffering or virtually no suffering.**	O	20	
A manipulation or use that causes stress, or pain, of a minor intensity for a short duration. **Little suffering.**	A	30	
A manipulation or use that causes stress, or pain, of a minor intensity for a long duration, or of a moderate intensity for a short duration. **Moderate suffering.**	B		
A manipulation or use that causes stress, or pain, of a moderate intensity for a long duration, or of a severe intensity for a short duration. **Severe suffering.**	C		
A manipulation or use that cause stress, or pain, of a severe intensity for a long duration, or of a very severe intensity for any duration. **Very severe suffering.**	X		

7. Expected Date of Completion: April 2004

ANIMAL DISPOSITION/FATE AT CONCLUSION OF EXPERIMENT/TEACHING EXERCISE ETC OUTLINED IN THIS PROTOCOL

The data in boxes 8 to 10 refer only to the animals noted in this protocol which actually entered the project and were manipulated - they do not refer to those it was proposed to manipulate but which were never used. This information is to be provided only when the experiment/teaching exercise has been completed and the animals have been disposed of as below.

8. Alive	Used	
Retained by your institution's farms/colonies	a	
Returned to commercial farmers	b	
Released to the wild	c	
Disposed of to others	d	
TOTAL ALIVE	=B=	

9. Dead	Used	
Killed for dissection, sampling, taking organs	a	
Died/destroyed in the course of the manipulation/use	b	
Euthanased after manipulation or use	c	
Died/destroyed for reason not associated with manipulation/use	d	
TOTAL DEAD	=C=	

10. GRAND TOTAL MANIPULATIONS/USED = B + C =

Check on the final return that B + C = A in the "Used" column of Box 2.

BOX 1: ANIMAL TYPE CODES:

Animal Type	Code
Rodents	1 a = Mice
	1 b = Rats
	1 c = Guinea Pigs
	1 d = Hamsters
Rabbits	1 e = Rabbits
Farm Animals	1 f = Sheep
	1 g = Cattle
	1 h = Goats
	1 j = Deer
	1 k = Pigs
Other Domestic Mammals	1 m = Horses
	1 n = Dogs
	1 o = Cats

Animal Type	Code
Birds	1 p = Fowls, Chickens
	1 q = Pigeons
	1 r = Other Birds
Miscellaneous	1 s = Marine Mammals
	1 t = Possums
	1 u = Reptiles
	1 w = Amphibia
	1 x = Fish
	1 z = Octopus, Squid, Crabs, Lobster, Crayfish
Other	1 y = Other Species (*name)