ASSESSMENT AND ALLEVIATION OF CASTRATION DISTRESS IN LAMBS.

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ABSTRACT

There is increasing pressure on the farming community to assess and minimise the distress caused by husbandry procedures. This is due to an increase in awareness of animal welfare throughout New Zealand, and economic pressures from overseas. This study involved an investigations into the acute pain-induced distress of lambs caused by castration, the effectiveness of different anaesthetic methods to alleviate that distress, characterisation and validation of behavioural responses as indices of pain-induced distress, and the assessment of the use of the burdizzo to reduce the acute pain-induced distress caused by ring castration. The castration techniques examined were ring, burdizzo, and ring + burdizzo. Short scrotum creation was also assessed. The alleviation techniques were injections of local anaesthetic into the scrotum, spermatic cords, testes or the scrotum + spermatic cords, 15 minutes prior to castration.

This study assessed the effectiveness of using a burdizzo before application of a rubber ring to reduce the distress, as indicated by cortisol and behaviour responses, caused by ring application. The concept of using a burdizzo to reduce the distress caused by ring castration was based on the hypothesis that 'disabling the afferent nerves from the testes would prevent nociception caused by ring application from being transmitted' (Kent et al. 1993,1995). It was found that the burdizzo used in the conventional manner (one application to each spermatic cord and the surrounding scrotal tissue with no overlap of 'cuts') together with ring application did not reduce the cortisol or behavioural responses to ring castration. Hence it is unlikely that pain will have been reduced.

This study characterised the cortisol and behavioural response to burdizzo castration. The cortisol response was found to have a duration of 180 minutes with a magnitude similar to that exhibited by ring lambs. However, the values remained elevated for longer than ring in lambs. Although the cortisol response is likely to indicate noxious sensory input caused by burdizzo application, it cannot be proved, using the results of this study, that sensory-independent

stimulation of the hypothalamic pituitary adrenal axis is not responsible for this cortisol response to burdizzo castration.

Local anaesthetic administrated into the scrotum, spermatic cords plus scrotum, or testes was found to abolish the cortisol response and either reduce or abolish different behaviours after ring castration suggesting that pain-induced distress caused by application of a rubber ring was prevented. The cortisol response and some behaviours caused by burdizzo plus ring castration were also prevented by injecting local anaesthetic into the scrotum prior to treatment indicating that pain-induced distress was also prevented. Local anaesthetic injected into the spermatic cords reduced numerically (although not significantly), but did not abolish the cortisol response to ring or burdizzo plus ring castration. This suggested that the scrotum, which was presumed to be unanaesthetised, was a significant source of nociception after these two treatments.

Local anaesthetic injected into the scrotum or spermatic cords did not reduce the cortisol or behavioural responses to burdizzo castration. This can lead to two conclusions; either local anaesthetic did not anaesthetise all the tissue effected by the burdizzo, or something other than sensory input stimulated the hypothalamic-pituitary-adrenal axis after burdizzo castration. Intuitively, the former seems most likely.

Some behaviour parameters were found to be useful when comparing the pain-induced distress caused by similar treatments that elicited similar behaviours, but not when comparing between treatments that caused different types of tissue damage and elicited different behavioural responses. Burdizzo castration did not cause any obvious abnormal behaviours, suggesting that either no significant sensory input was caused by burdizzo application or that our behaviour observation methodology was not sensitive enough to pick up nuances of behaviour. Hence it was not possible to use behaviour to compare intensities of pain-induced distress caused by ring or burdizzo castration.

Practically the use of the burdizzo in the conventional manner to reduce the pain-induced distress caused by ring application cannot be advised. Further

work needs to be done to assess practical aspects of the modified use of the burdizzo (across the whole width of the scrotum suggested by Kent et al. 1993,1995) before it can be recommended as an alternative method of castration.

The use of local anaesthetic in the field needs to be investigated further before it can be recommended. It seems that simple methods of local analgesia may be possible, however the danger of possible complications such as sepsis must be evaluated.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 ANIMAL WELFARE

The relationship between humans and animals evident at the beginning of human history was a complex of reciprocal predation and competitive scavenging. This relationship has been modified, and the context changed from hunting and scavenging to extensive and intensive farming practises. Moreover, human association with animals has diversified to include other relationships such as companion, worker and experimental subject. These relationships usually result in net benefit for humans in terms of sustenance, knowledge, economics and/or pleasure.

As human relations with animals have developed, our understanding of them and their needs has changed. Until relatively recently, many humans believed that they had no obligation to animals other than keeping them alive for human use. This attitude resulted from an acceptance that animals did not suffer in the same way as humans and was supported by the notion that animals lacked "soul" (Uvarov 1984). Today we believe that animals experience pain, discomfort, fear and distress and we only have to watch some of our pets, to realise that animals can experience happiness and frustration in a fashion somewhat similar to humans. As our understanding of what an animal can experience increases, so our concern for the animals under our care grows.

The majority of humans do not like to inflict pain, discomfort or suffering on other people. The more we discover how similar human beings and animals are with respect to suffering, the more people are concerned with animal welfare and increasingly the consideration given to animals is approaching that given to other human beings.

This rise in animal welfare consciousness which affects our attitudes and behaviour to animals is categorised by Hurnik (1988) as follows;

a) application of moral standards to actions that fall within the perceived sphere of personal responsibility;

b) protection of those who cannot effectively protect themselves; and

c) prevention of suffering which is preventable.

Stafford (1995) pointed out the apparent conundrum of increasing animal welfare/rights activity whilst society becomes increasingly apathetic towards homeless and other needy humans. This raises many questions, for example does the redirection of the behaviours categorised by Hurnik (1988) result from despair in human worth or from a desire to achieve self justification or purpose from animal protection (Stafford 1995)?

Although the first animal welfare concerns and legislation were centred on deliberate cruelty to animals such as bull baiting (Uvarov 1984, Rollin 1991), more recently concern at the way animals are treated on farms and in laboratories has increased.

The people holding the strongest animal rights/welfare views are often members of urban based populations, having little or no contact with the animal production systems themselves. Books such as Ruth Harrisons' *Animal machines* (1964) and Peter Singer's *Animal Liberation* (1976) and exposure to the views of strong animal rights groups, further influence societal concern for animal welfare.

Because of the increasing interest in animal welfare by humans, a number of animal husbandry systems and procedures have been assessed and attempts have been made to evaluate the welfare advantages and disadvantages of each.

Definitions of Animal Welfare

A dictionary definition of welfare includes the condition of health, happiness and comfort (Collins Pocket English Dictionary, 1984). This definition is not detailed

enough to define conditions that indicate a state of good welfare but includes the concept of a satisfactory mental and physical state (Dawkins 1980).

Researchers have developed definitions of welfare or well-being specifically for animals, for instance "animal well-being is the state or condition of physical and psychological harmony between the organism and it's surroundings" (Hurnik 1988). However, definition of "harmony" is difficult and so, in practical terms, the Hurnik definition is not very useful. Other definitions emphasise the animal's "state as regards its attempts to cope with it's environment" (Fraser and Broom 1990) or it's "physical ...and mental well-being and the absence of disease and injury" (Dawkins 1980).

Definitions of animal welfare usually denote an absence of suffering or it's major components (anxiety, fear, pain and distress) (Mellor and Reid 1994). An animal's well-being or welfare can be considered on a continuum between high welfare and severe suffering (Mellor and Reid 1994).

A practical way of approaching animal welfare is the "five freedoms" concept first proposed by the Farm Animal Welfare Council (FAWC) in the United Kingdom in 1979. These "freedoms" are a guide to providing for the animal's needs and focus more effectively on "needs" than do other definitions which emphasise well-being as the absence of suffering.

The 'freedoms' are listed below.

1. **Freedom from hunger and thirst** - achieved by ready access to fresh water and a diet to maintain full health and vigour.

2. **Freedom from discomfort** - achieved by providing an appropriate environment including shelter and a comfortable resting area.

3. **Freedom from pain, injury or disease** - achieved by prevention or rapid diagnosis and treatment.

4. **Freedom to express normal behaviour** - achieved by providing sufficient space, and proper facilities and company.

5. **Freedom from fear and distress -** achieved by ensuring conditions and treatment which avoid mental suffering.

These freedoms have since been revised both by the FAWC and by others (Mellor and Reid, 1994). The latter reworked the five freedoms into domains of potential welfare compromise and included the major components of suffering (anxiety, fear, pain, and distress) into what was previously freedom five. This was done because the freedoms, although goals to strive for, were often not achievable. When there is no or very little welfare compromise in all domains, ie the animal's nutritional, environmental, health, behavioural and psychological needs are met, then a state of good welfare is obtained.

Further discussion in this chapter on meeting the "needs" of animals will use the "domains of welfare compromise" (Mellor and Reid 1994) as a framework.

1.2 ASSESSMENT OF WELFARE

With increasing public demand for every aspect of animal production to be assessed and modified to minimise welfare compromise, there need to be good measures of animal welfare. However, due to the complexity of animal existence and experience, the large numbers of different species and breeds, the different living conditions and circumstances, there is no one simple way of assessing animal welfare.

In the earlier days of animal welfare awareness, good welfare was often equated with good animal production. However, this is now considered to be unacceptable because within a group of highly productive animals the welfare of individuals can be poor (Dawkins 1980). Modern technology can overcome problems that are manifest in production parameters, while still compromising animal welfare. The five "domains of welfare compromise" provide good guidelines for assessing individuals in different circumstances whilst more specific indices of measurement for each domain are being developed. Mellor and Reid (1994) provided a grading system for their "domains of welfare

compromise" that although designed primarily for laboratory animals, is generally applicable to any animals for which humans are directly responsible.

1. **Thirst, hunger and malnutrition**. Behavioural studies can determine the motivational state of an animal to drink or eat. Biochemical methods of assessing hydration status and nutrient deficiencies are available. Standard nutritional requirements of water, protein, carbohydrates, fats, vitamins and minerals are known for most domestic species (Agriculture Research Council 1980; National Research Council 1980). Indices such as body condition scoring can be used to assess the nutritional status of an animal. Abnormal behavioural and clinical symptoms can indicate specific nutrient deficiencies (Mellor 1992).

2. **Environmental challenge.** Behavioural studies can provide the most obvious indication of discomfort or exposure. Preference testing has been used to "ask" animals to choose between surroundings or enclosures (Duncan 1978; Dawkins 1980). These studies have some limitations. An animal may not necessarily choose what is best for itself, ie. avoiding the veterinarian or eating poisonous plants (Stafford, 1995). An animal may choose one surrounding or circumstance for 60% of the time but the 40% of the time spent in the other circumstance may be very important to the animal. More recent studies use the economic theory of supply and demand to determine how much a condition or commodity is "worth" to an animal (Matthews 1993, Dawkins 1983,1990). An animal can be trained to work, eg. pushing a nose plate, for a reward such as straw or the ability to see a mate. The amount of work an animal will perform is thought to be related to the perceived importance of that reward or "commodity" (Matthews 1993). Physiological parameters such as heart rate, shivering, metabolic and sweating rates, and prolactin levels are used for evaluating the degree of exposure (Mellor 1992).

3. **Disease, injury and functional impairment.** The veterinary profession is concerned with the diagnosis, prevention and treatment of injuries and disease. However, it is important that persons responsible for animals to

recognise the clinical symptoms of injuries or disease so that veterinary expertise can be used. It is also important for the farmer or animal handler to use husbandry practises that prevent disease or injury.

4. **Behavioural/interactive restriction**. The presence of abnormal behaviours, such as stereotypies in sows held in stalls (Lawrence and Terlouw 1993), or wool pulling in sheep fed a concentrated diet and unable to ruminate (Stafford 1988) are indicators of a restricted environment. In some species, the inability to perform some behaviours may result in injury because of redirected behaviours such as tail biting in pigs or flank sucking in calves.

5. **Anxiety, fear, pain and distress.** As these components of suffering are subjective experiences, it can be difficult to assess whether an animal is suffering and to what extent. Although generally acknowledged, it is (and will probably remain) unproven whether animals can experience any of these emotions. However, the physiological and behavioural criteria that lead to the judgement that a human being is experiencing anxiety, fear, pain, or distress can be generalised by analogy with substantial measure of agreement to other animals (Bateson 1991, Petrie 1994).

1.3 PAIN, STRESS, DISTRESS AND SUFFERING

The concepts of pain, stress, distress, and suffering are broad and are the focus of much discussion. This section will focus on these concepts in relation to the management practise of castration. The effects of castration (eg. ring or burdizzo application) can be broadly placed into three categories. These are; the stimuli, the perception and modulation of the stimuli (eg. brain), and the response/s to the stimuli.

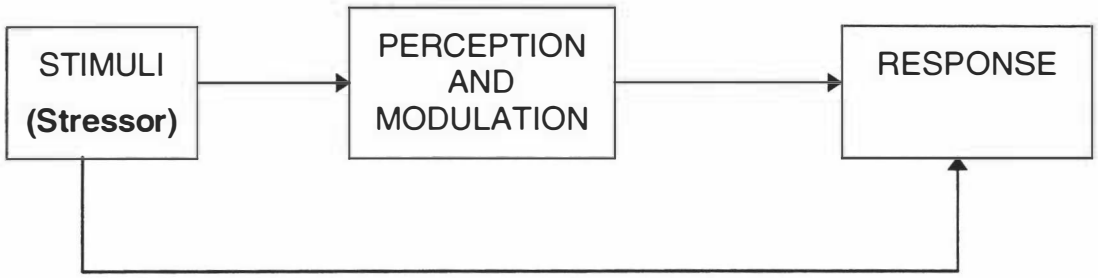


Fig 1.1 The three aspects of an animal's reaction to a stimuli such as castration.

1.3.1 Pain

The stimuli

When tissue is damaged by a stimulus such as physical trauma, receptors on the end of nerves called nociceptors become active. These receptors increase the activity of their afferent nerves that synapse with the spinal cord. Nociceptors may be stimulated by pressure, heat and chemicals which are above certain thresholds (Headley 1993).

Nociception (nociceptor afferent nerve activity), when it reaches the spinal cord may result in a spinal reflex such as movement of a limb away from the source of stimulation, and/or continued transmission of nociception to the brain. Transmission of nociception along the spinal cord involves the activation of spinothalamic tract neurons. The amount of stimulation of the spinothalamic tract neurons can be modulated in the dorsal horn of the spinal cord by activity of large and small afferent fibres (Kimble 1988, Cervero and Laird 1991).

Castration by ring application or burdizzo causes nociception due to the damage of scrotal, testicular and spermatic cord tissues. Nociception, if registered consciously, can result in pain which itself can elicit other physiological responses, some of which are used to characterise stress. If a

stimulus such as physical trauma results in a stress response then it is called a **stressor**. Other stimuli, which are perceived by sensory systems other than nociception, such as visual, olfactory, tactile or auditory processes, may also cause stress responses and hence be termed stressors.

Perception and it's modulation

Pain has been defined by the International Association for the Study of Pain as "an unpleasant noxious sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Headley 1993) or " a sensory experience which is itself aversive" (Fraser and Broom 1990). The important point of these definitions is that 'pain' is the sensory and/or emotional experience that results from the perception of nociception as being noxious. This process of perception involves many parts of the brain which are stimulated by tracts from the spinal cord which are in turn stimulated by afferent nerves from nociceptors.

It is likely that as well as areas in the cerebral cortex, which discriminate and interpret pain (Ganong 1989), other structures such as the thalamus, and parts of the limbic system will modulate perception of nociception (Headley 1993). Parts of the brain such as the amygdala, hippocampus, septal nuclei and parts of the hypothalamus may differentiate between negative and positive stimuli, and may also be involved in the affective and motivational components of pain (Kimble, 1988). Furthermore, the perception of pain can be modified by emotional states because of the involvement of the amygdala (Livingston and Waterman, 1992). Indeed, physiological and other responses can be initiated from emotional stimuli such as fear and anxiety that are not a result of nociception. Modifications of the perception of nociception can result from factors such as previous experiences of the individual, genotype, sex and age. Complicating factors such as types of pain (visceral, deep, superficial, muscular), different pain thresholds, and stress-induced analgesia, as well as the effects of placebos, hypnosis and acupuncture, all can alter the pain experience (Stafford, 1995). Stress-induced analgesia (eg. on the battlefield) is

a phenomenon that is seen in wounded individuals who are having a powerful emotional experience such as extreme fear and who do not feel pain from, or even notice a severe wound (Amit and Galina 1986).

Can animals experience pain? Humans can describe in detail their experience of pain so that detailed knowledge of the human pain experience and the factors that affect it has been documented. Because of the inability of humans to understand animal communication in depth, animal experiences of pain will probably never be fully understood. The question of animal pain has led to much discussion and research. It is likely that non-human mammals experience pain in a similar way to humans. This is because of a number of similarities between mammals and humans. According to Bateson (1991) these similarities include;

1. possession of receptors sensitive to noxious stimuli, located in functionally useful positions on or in the body;

2. possession of brain structures analogous to the human cerebral cortex;

3. possession of nervous pathways connecting nociceptive receptors to higher brain centres;

4. possession of receptors for opioid substances found in the central nervous system, especially the brain;

5. analgesics modify response to noxious stimuli and are chosen by the animal when the experience is unavoidable;

6. responds to noxious stimuli by avoiding them or minimising damage to the body;

7. animal learns to associate neutral stimuli with a noxious stimulus.

When humans and animals experience an emotion such as pain, fear or anxiety, this may result in one or more responses that enable the animal to cope with the cause and with the resultant emotion.

The Responses

The responses to pain can be physiological, behavioural, immunological, or pathological and may be reflected in productivity.

Of the physiological responses to nociception and other stressors, two are especially important. The first, a very quick response, is the "fight or flight" reaction first described by Cannon (1929) which involves the release of adrenaline from sympathetic neurones in the adrenal medulla. Adrenaline increases both the rate and force of contraction of the heart, causes vasoconstriction in the major organs and vasodilation of skeletal muscle, stimulates glycogenolysis, produces a prompt rise in metabolic rate, and increases alertness (Ganong 1989). These effects of adrenaline prepare the animal mentally and physically to either defend itself or remove itself from the source of pain or stressor.

The second main physiological response to nociception or other stressors, is embodied in Selye's "General Adaptation Syndrome". This slower and more prolonged response to a stressor involves hormones from the hypothalamus, anterior pituitary and adrenal cortex (Selye 1956). This mechanism seems to enable animals to cope with acute and chronic stressors by helping to mobilise the body's defence mechanisms in preparation for exertion and tissue repair (Ewbank 1992).

The extent to which both of these physiological mechanisms are stimulated is assumed to be in direct relation to the extent to which the stimulus is perceived as noxious. It is realised that these two responses are not the only physiological responses to stressors. Other mechanisms of transmitting information to the rest of the body to initiate responses, include the release of oxytocin and vasopressin from the neurohypophysis of the hypothalamus, and activation of the somatomotor system (Ganong 1989).

1.3.2 Stress

The term '**stress**' in common usage in human context, refers to the physiological and behavioural responses to stressful circumstances (stressors) such as work or family pressure (Stafford 1995). In the context of animals, there have been many different ways of thinking about and defining stress. Stress has been defined as the integration of the stimulus (stressor) and the measured response, and takes account of the individual differences between animals (Ladewig *et al.* 1993, Stafford 1995). Another opinion is that stress can be thought of as the largely adaptive response of an animal to a noxious or potentially noxious stimulus (stressor) (Ewbank 1992).

The psychological component of the stress response (perception and modulation) is confused by a physiological stress response exhibited when perception is unlikely. This is demonstrated when unconscious animals under general anaesthesia, exhibit a physiological response such as elevated cortisol levels to surgery. This has been seen in sheep (Pearson and Mellor 1975), goats (Pearson and Mellor 1975), and dogs (Frank *et al.* 1992; Fox *et al.* 1994). A differentiation has been made between stress responses that are either independent of, or, influenced by an animal's psychological state. The term 'Stress' has been used as the encompassing term for the physiological response without perception and modulation by conscious processes, and the term distress has been used to describe a stress response to aversive or noxious stimulation in an animal that is fully conscious and is able to perceive the stressor as noxious (Lester 1991). However it may be unnecessary to make this distinction as in the majority of cases a stress response is likely to be influenced by psychological processes.

In this study the term 'stress' is used to embody the physiological response to a stressor whether it is influenced by psychological factors or not. In this definition, stress encompasses the perception of and adaptation (by physiological, immunological, behavioural or production means) to stressors. This definition does not encompass the stressor.

It is understood that there are varying degrees of intensity and duration of the stress response, and this can be recognised by using prefixes such as mild, moderate, or marked. There is however a problem of determining when a stress response is mild, moderate or marked. Determination is likely to result from subjective judgements based on knowledge of the normal variation and limitations of the physiological parameter studied. It is also likely to be influenced by human experience of analogous situations.

Distress

The term "**distress**" has been used to describe the level of stress which occurs when an animal cannot cope with a stressor and the stress response becomes damaging to the animal and is unadaptive (Lynch *et al.* 1992). The response an animal exhibits to a stressor will vary with the intensity and duration of the stressor (Ladewig *et al.* 1993). When the intensity and duration of a stressor exceeds the animal's ability to cope, the animal enters a pre-pathological state and if the stimulus continues, develops immunological deficiencies and pathological conditions such as stomach wall lesions (Wiepkema and Koolhaas 1993). When the stressor is of an intensity and duration that the animal enters a pre-pathological state, then the animal is distressed or overstressed (Stafford 1995).

A limitation to this definition of distress is that it can be difficult to determine when an animal is not coping with a stressor or is in a pre-pathological state.

An alternative definition of distress embodies the physiological response to a stressor that is influenced by the psychological state of the animal (Lester 1991). This also has its limitations. For example, when an animal is being handled, a small physiological response, such as increased adrenaline levels or even corticosteroids may be exhibited. Does this indicate that an animal is distressed?

In this thesis, distress will be used to describe the physiological state an animal is in when its adaptation mechanisms are unable to adapt to a stressor and return the animal to an unstressed state. Stress states may be influenced by

physical factors such as nociception, but also by psychological and emotional factors such as fear, anxiety or even boredom. It is recognised that psychological and physical factors can interact as prior components of physiological responses to noxious stimuli. It is also acknowledged that the point at which marked stress becomes distress is very difficult to define. However, in cases where an extreme physiological response is exhibited, it is assumed that the animal is in a state of distress. In the case of castration and other tissue mutilations, it is assumed that for the duration of the corticosteroid response when corticosteroid values are increasing and the animal is attempting to maintain homeostasis, pain-induced distress is being experienced.

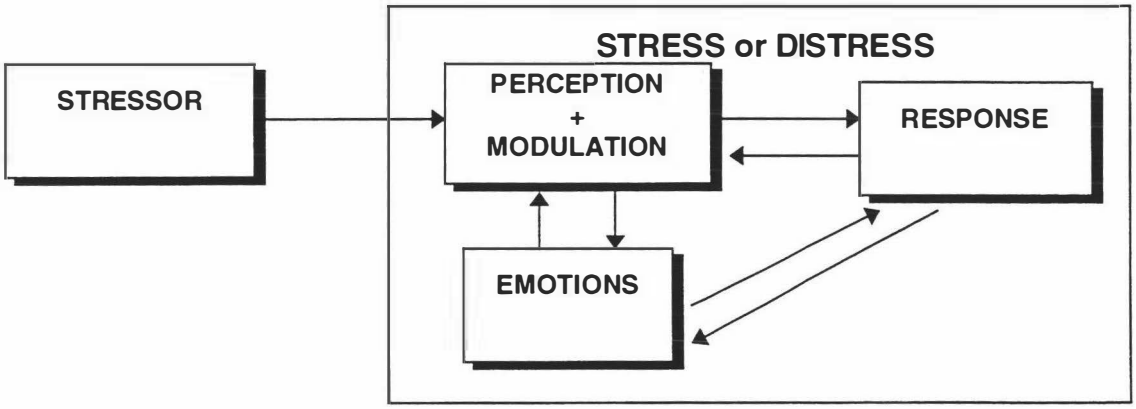


Fig 1.2 The boundaries of stress and distress.

Suffering

The use of the term 'suffering' in animal welfare science has been criticised as it can evoke strong emotions in lay people and scientists. Because of its emotive content, 'suffering' has been avoided by some scientists (Orlans 1993). The use of the term 'suffering' and what it describes is however, very important in the study of animal welfare.

The American Veterinary Medical Association declares suffering to be a 'highly unpleasant emotional response usually associated with pain and distress' (Lynch *et al.* 1992). "Suffering occurs when unpleasant subjective feelings are

acute or continue for a long time because the animal is unable to carry out actions that would normally reduce risks to life and reproduction in those circumstances" (Dawkins 1990). "Essentially suffering is an unpleasant emotional state brought about by physical pain/distress and/or psychological distress" (Stafford 1995). The main component of all these definitions is the "negative emotional state". Suffering is used in this thesis as a term that encompasses all the negative emotions such as fear, anxiety, pain, anger, frustration, or boredom that can lead to a distress response (Fig 1.3). For example if an animal is experiencing a negative emotion such as fear and it results in an extreme physiological stress response (distress), it can be said to be suffering.

There is debate over whether consciousness, and hence the presence of self-awareness, is an important prerequisite for the experience of suffering (Stafford 1995). Mellor and Reid (1994) stated that "it is axiomatic that without sentience there cannot be any feeling by the senses and without consciousness, sentient animals cannot suffer". Whether an animal is aware of it's self or not may be the subject of much research and discussion, but may well be never determined. However, if an animal can experience pain and other emotions such as anger and frustration, and these result in distress, it must be able to suffer (Stafford 1995).

There are however several conundrums. Is an animal that is deficient in some form of nutrient, but not necessarily experiencing any of these negative emotions, suffering? And if not, is that deficiency detrimental to the animal's welfare?

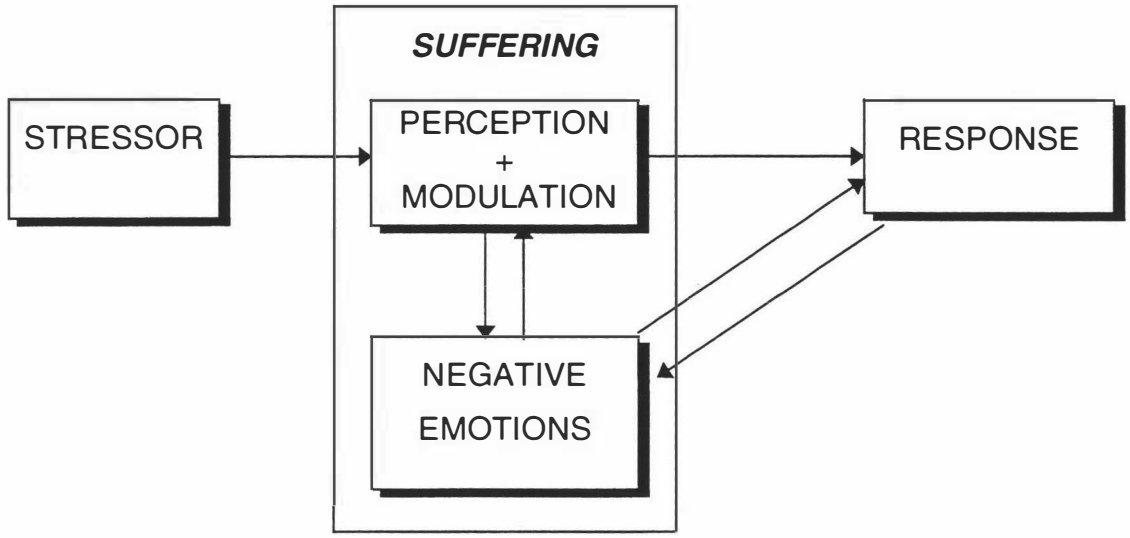


Fig. 1.3 The boundary of Suffering

1.4 DISTRESS IN RELATION TO HUSBANDRY PROCEDURES INCLUDING TISSUE REMOVALS

Increased awareness of animal welfare issues both in the New Zealand and the international marketplace has required that animal husbandry methods be assessed to determine the extent of welfare compromise (Bayvel 1992; Baddely 1992).

"Animal welfare considerations are becoming increasingly important for the keeping and farming of animals, both in New Zealand and internationally. Practises which may once have been deemed acceptable are now being reassessed and modified according to new knowledge and changing attitudes. High standards of animal welfare are not only important legally but also have direct economic benefit and ensure direct market access" (MAF 1991).

Many husbandry procedures have been assessed for welfare compromise using physiological and behavioural measures of stress or distress. Procedures assessed include:

- **Cattle**; dehorning (Petrie *et al.* 1996b; Sylvester *et al.* 1993; Stafford and Mellor 1993), castration (Fell *et al.* 1986; Cohen *et al.* 1990; King *et al.* 1991; Mellor *et al.* 1991; Robertson *et al.* 1994), tail docking (Petrie *et al.* 1995, 1996a) and branding (Lay *et al.* 1991).

- **Sheep**; castration (Shutt *et al.* 1988, Mellor and Holmes 1988, Mellor and Murray 1989a,b; Lester *et al.* 1991a,b,; Mellor *et al.* 1991; Kent *et al.* 1993, Molony *et al.* 1993), tail-docking (Mellor and Murray 1989a,b; Lester *et al.* 1991a; Mellor *et al.* 1991), teeth grinding (Spence *et al.* 1986, Denholm and Vizard 1986a,b), electroejaculation (Stafford 1995) and shearing (Kilgour and De Langen 1970; Purchas 1973; Fulkerson and Jamieson 1982; Hargreaves and Hutson 1990).

- **Deer**; velvet removal (Pollard *et al.* 1992; Matthews *et al.* 1994), electroimmobilisation (Stafford and Mesken 1992).

- **Pigs**, housing (Barnett *et al.* 1985, 1987b, 1989, 1991b), castration (White *et al.* 1995).

1.5 MEASURING DISTRESS ASSOCIATED WITH TISSUE REMOVAL.

Physiological and behavioural parameters have been used in an attempt to quantify the acute stress or distress experienced by farm animals after husbandry procedures that cause tissue damage. The main physiological parameters used include the plasma concentrations of hormones involved in Selye's 'General Adaptation Syndrome'(GAS) which are released by tissues involved in the hypothalamic-pituitary-adrenal axis (HPA). Other parameters that have been used include heart rate and catecholamine levels.

1.5.1 The Hypothalamic-Pituitary-Adrenal Axis (HPA)

For an animal to experience a stimulus as a stressor, information about the stimulus from the senses (visual, auditory, olfactory, tactile or nociception)

must be processed by the central nervous system (CNS). If the stimulus is then perceived as noxious or potentially noxious, a physiological response may be initiated. One response is initiated by stimulation of the hypothalamus probably by neurones projected from the cerebral cortex. Stimulation of the hypothalamus results in the release of corticotropin releasing factor (CRF) which is synthesised and released by neurones in the hypothalamus. CRF is transported to the anterior pituitary via the hypothalamic-pituitary portal blood vessels where it stimulates the release of adreno-corticotropic hormone (ACTH). ACTH and b endorphin are both synthesised in the anterior pituitary from the precursor molecule pro-opiomelanocortin (POMC) and are released concomitantly (Guillemin *et al.* 1977). ACTH released into the circulatory system stimulates glucocorticoid secretion from the adrenal cortex. The main glucocorticoid secreted from the adrenal cortex in sheep is cortisol. Cortisol has been used routinely as a measure of acute stress or distress in studies assessing husbandry techniques that result in tissue damage.

There are, however, limitations to using cortisol as a parameter to measure distress. Rushen (1986) has argued that increases in plasma corticosteroid concentrations in animals may not reflect stress or suffering. He argued that plasma levels of corticosteroids are not sensitive to variations in painfulness of treatments such as electric shocks to the feet of rats. Furthermore, as Lester (1991) and Petrie (1994) point out, the stress rats experience while being handled and placed in a situation where they cannot escape, such as in the experiment described by Rushen, may be as stressful as the electric shocks themselves and may thereby obscure responses to the shocks.

In addition, Rushen (1986) questions the use of plasma corticosteroid concentrations as a measure of stress or distress on the grounds that it is not only situations which are presumed to be distressful that increase corticosteroid concentrations. He cited studies that have shown that events such as coitus (Szechtman *et al.* 1974), voluntary exercise (Sutton and Casey 1975) and regular deliveries of food which are presumed not to be distressful, increase plasma corticosteroid concentrations. However, Rushen's (1986) rejection of corticosteroids as a useful indicator of distress does not recognise that it is not

necessary for all corticosteroid release to be stressor induced for corticosteroid concentrations to be useful as a stress indicator. It has been shown that plasma corticosteroid concentrations do increase under presumably stressful or distressful circumstances and they have been useful in comparing stress or distress elicited by different treatments (Purchas 1973; Shutt *et al.* 1988; Mellor and Murray 1989a,b; Lester *et al.* 1991a,b; Mellor *et al.* 1991; Apple *et al.* 1993; Kent *et al.* 1993; Weeding *et al.* 1993; Kent *et al.* 1995).

The usefulness of corticosteroid concentrations as an indicator of stress responses to painful experiences is supported by the abolition of the cortisol and behavioural responses by local anaesthetic which removes nociceptor input from damaged tissues (Boandl *et al.* 1989; Wood *et al.* 1991; Petrie *et al.* 1996b; C.M. McMeekan, D.J. Mellor, K.J. Stafford, R.B. Bruce, R.N Ward, unpublished data). Although measuring corticosteroid concentrations is a useful method of comparing the stress or distress being experienced, care must be taken to control for psychological and physical stressors such as handling and blood sampling. Situations where activities such as coitus and active food consumption may produce false indication of stress or distress must also be avoided (Herd 1989). Finally, cortisol concentrations can only indicate the *relative* intensity of stress or distress experienced. Judgement on the *actual* degree of stress or distress indicated by the cortisol response can only be subjective as can the judgement of whether an animal is markedly stressed or distressed.

1.5.2 Behavioural measures of stress or distress.

There are many behavioural indicators of stress or distress. A stressor to one species of animal such as the sheep may elicit active avoidance behaviour when the same stressor to another species, such as the dog may result in attack behaviour. Species of the same family may also exhibit different behaviours when confronted with the same stressor. For example, goats when escaping a predator will climb cliffs, putting obstacles and insecure terrain between themselves and the predator (Kilgour and Dalton 1984; Lynch *et al.* 1992). Sheep, which are gregarious animals, when threatened by a predator

will run and clump into a cohesive flock (Kilgour and Dalton 1984; Lynch *et al.* 1992).

The behaviour exhibited by members of the same species to apparently similar stressors, will often vary. For example, lambs that have been castrated and tailed with a ring, lie down (laterally or ventro-laterally) and writhe and kick in the first hour after treatment (Molony *et al.* 1993; Lester *et al.* 1996). However, lambs that have been castrated and tailed surgically, often remain standing quite still (Lester *et al.* 1996; Molony *et al.* 1993). One interpretation of this difference in behaviour is that ring castration causes much more stress or distress than surgical castration. However, both surgical and ring castration methods have been shown to elicit a significant plasma cortisol response and are both thought to cause significant amounts of distress. Indeed, the cortisol response to surgical castration is far greater in magnitude and duration than is the response to ring castration, suggesting that the distress caused is greater with surgery. Thus, such qualitative differences in behaviour may be misleading with regard to quantitative differences in the degree of distress experienced (Lester *et al.* 1996). Furthermore, the presence or absence of different types of behaviour cannot necessarily indicate if distress is indeed being experienced.

Measurement of behaviour is useful in observing the duration of distress and in some cases comparing the intensity. For behaviour to be used to compare the intensities of the distress caused by two treatments, there needs to be a validating continuity between the two situations, with the behavioural responses in both being part of the one continuum (Lester *et al.* 1996). For this to occur, the stimuli have to be of a similar nature. Behaviour is often used in conjunction with plasma cortisol concentrations to provide a more accurate representation of the duration and (with care) the intensity of distress experienced by animals (Shutt *et al.* 1988, Mellor and Murray 1989a, Lester *et al.* 1991a,b; Molony *et al.* 1993, Kent *et al.* 1995).

1.6 CASTRATION

1.6.1 Reasons for Castration

Castration has been a common agricultural practise for millennia. It is used to reduce aggression, reduce meat taint (especially in boars and billy goats), improve the flavour of meat (usually by increasing fat content), increase the ease of handling stock (sheep, cattle, pigs and deer), prevent indiscriminate breeding (sheep, cattle, pigs and deer), remove the long scrotum of sheep (for easier meat works processing), and prevent male behaviour/sexual activity reducing the proliferation of sexually transmitted diseases and injuries to individuals or cospecifics (Wood and Molony, 1992).

In some farming systems it is now becoming common to leave males entire. Husbandry systems have been devised for the management of uncastrated animals. Lambs that are uncastrated reach growth targets faster and more efficiently than castrated lambs (Probert and Davies 1986), and have leaner carcasses (Wood and Molony 1992).

A significant proportion of cattle in New Zealand are now left entire and successful methods of raising bull beef from pasture have been developed (McCrea and Morris 1984). About 500,000 entire bulls are slaughtered annually in New Zealand representing 16 % of New Zealands total beef production (Morris *et al.* 1994)

Ram lambs can be rendered infertile and the pendulous scrotum reduced without actually castrating them by using the "short scrotum" technique. With this method the testes are pushed up against the abdominal wall using a rubber ring which is placed on the scrotum distal to the testes. The lower part of the scrotum becomes ischaemic and consequently sloughs off. The increased temperature of the testes inhibits spermatogenesis whilst still enabling testosterone production. This produces an animal that is infertile while still retaining all the advantages of testosterone production, such as increased growth rate and lean muscle production (Probert and Davies 1986).

Nevertheless, farmers continue to castrate lambs, so that it is necessary to evaluate the castration procedures available to enable recommendations to be made that are both acceptable to farmers and beneficial to the welfare of animals.

1.6.2 Methods of Castration

Methods of castration that are currently used on farms include;

- **Surgery** (lambs and calves). This method is not recommended with lambs for welfare reasons (Lester *et al.* 1991a,b) but is still often done. The lower part of the scrotum is removed using a sharp knife. The testes are then individually grasped and pulled out by hand or with a pliers like instrument or, on occasion, the farmers teeth. The pulling action breaks the spermatic cord, usually within the inguinal canal. Although this can be a quick method of castration, the chance of sepsis is increased as an open route of infection is created (Wood and Molony 1992). The success rate of this procedure is 100 %.

- **Rubber Ring** (lambs). A tight rubber ring is applied to the neck of the scrotum, proximal to the testes, using an elastrator. This causes ischaemia of the scrotum and testes which slough off after some time. This method is very easy and quick to perform. There is little immediate risk of infection. However as the scrotum breaks down pathogens can gain access to living tissue near the ring. The success rate of this method of castration is almost 100%.

- **Short scrotum** (lambs). A tight rubber ring is applied to the scrotum distal to the testes which are held firmly against the abdominal wall. This produces lambs with the same physical and behavioural characteristics of ram lambs but they are infertile. The success rate of sterilisation using this method is approximately 96% (Dobbie *et al.* 1985).

- **Burdizzo** or the **bloodless emasculator** (lambs and calves). A burdizzo, which is a clamp like device, is used to crush the scrotum and the underlying spermatic cord, causing ischaemia followed by atrophy of the testis. It is usually applied twice, each crush encompassing one of the two spermatic cords and the surrounding scrotal tissue. However, enough vascularised tissue must be left between the two crushes to prevent total ischaemia and sloughing of the scrotum. This method can be slower than the ring. The chance of infection is low if the skin remains intact. However, if the skin is broken or ischaemic necrosis occurs pathogens can gain access to live tissue (Wood and Molony 1992). The success rate of this method depends on the skill of the operator, how long the burdizzo is applied to each spermatic cord, and the quality of the burdizzo itself.

- **Immunisation** (sheep). Ram lambs can be immunised against testosterone or luteinizing hormone releasing hormone (LHRH). This method is not used under field conditions at present as more research is being undertaken into its effectiveness.

- **Chemical**. This method is sometimes used in cattle but is not used in sheep. A chemical solution which causes tissue necrosis is injected into each testis. Chemicals that have been used include α-hydroxypropionoic acid and formalin. The efficacy of this treatment is thought to be good if the procedure is performed properly and if time is taken to massage the testes to ensure thorough dispersion of the chemical (Cohen *et al.* 1990).

Analgesia or **local anaesthetic** is not used by farmers when castrating lambs under 1 week (Great Britain) or 9 weeks (New Zealand) of age. It is not practicable in the farming situation for reasons that include, the time taken, the number of needle penetrations increasing the risk of infection, and the cost of the local anaesthetic used. Nevertheless, it has been shown that extensive infiltration of local anaesthetic into the epidural space, scrotum, testes, and

spermatic cords can abolish behavioural and cortisol distress responses to ring castration plus tail docking (Wood *et al.* 1991).

1.6.3 Distress Associated with Castration.

The early evaluations of the distress caused by castration and tail docking, used production measures such as growth and healing rates in attempts to assess the distress caused by different methods (Garner and Sanders 1936; Barrowman *et al.* 1953,1954; Ewer 1942; French *et al.* 1994). No significant differences were found in production or in growth rates between lambs castrated and tailed by different methods. However, these reports, although providing some anecdotal evidence on differing types of behaviour immediately after different treatments, did not provide information on the degree of acute distress experienced by the lambs. In recent studies, however, attempts have been made to quantify the difference in degrees of distress experienced by lambs castrated by various methods with or without tail docking.

Castration practises that have been assessed for welfare compromise include the following.

Surgery: Surgical castration with or without tail docking caused a large cortisol response in sheep of all ages studied. This response lasted at least three to four hours (Lester *et al.* 1991a, Kent *et al.* 1993) and probably as long as eight hours (Lester *et al.* 1991b).

The distress caused by surgical castration and tailing has been compared with that caused by ring castration and tailing by Shutt *et al.* (1988) who, by reference to plasma b-Endorphin and cortisol concentrations, along with cursory behavioural observations, asserted that ring castration plus tailing is more distressful than surgical castration plus tailing. These findings were disputed by Mellor and Holmes (1988) and by Barnett (1988). When the cortisol concentrations obtained by Shutt *et al.* (1988) were reinterpreted by

Mellor and Holmes (1988), they concluded that ring castration plus tailing is less distressful than surgical castration and tailing. Later studies (Lester *et al.* 1991a,b; Kent *et al.* 1993) provide support for the revised conclusion.

Surgical castration has been shown to cause a large cortisol response in some calves. The distress when it occurs, inferred from the cortisol response, can last for up to 6 hours. Surgical castration has been shown to cause greater distress to calves than rubber ring castration (Fell *et al.* 1986, Robertson *et al.* 1994), burdizzo castration, (King *et al.* 1991, Robertson *et al.* 1994), or chemical castration (Cohen *et al.* 1990).

Ring: This method of castration with or without tail docking has received a large amount of attention from numerous researchers and can now be used as a standard for comparison between studies. Ring castration caused significant distress, inferred from cortisol and behavioural responses, for about 1.5-2.0 hours after treatment in lambs 1 week old (Mellor *et al.* 1991, Kent *et al.* 1993, Molony *et al.* 1993) and for about 3-3.5 hours in lambs 4-5 weeks old (Lester *et al.* 1991a, Kent *et al.* 1993, Molony *et al.* 1993). The cortisol response to ring castration alone was similar to the cortisol response to ring castration plus tail docking (Lester *et al.* 1991a).

In cattle the distress caused by ring application and other methods of castration seems to be influenced by a number of factors. These include age and the method of calf rearing. Mellor *et al.* (1991) found that ring castration of hand reared calves aged between 1 and 7 days produced little evidence of distress. However, this was in direct contrast to Robertson *et al.* (1994) who showed that calves of a similar age can exhibit a significant cortisol and behavioural response that lasted at least 2 hours after ring application. The difference between the two studies may have been that the calves used by Mellor *et al.* (1991) were isolated from other calves and their cow prior to and during the experiment, compared to those observed by Robertson *et al.* (1994) whose calves were kept in pairs prior to and during the study.

Burdizzo: The burdizzo, when applied for 10 seconds to each spermatic cord with it's associated scrotal tissue and to the tail of 5-6 day old lambs, elicited an immediate and prolonged cortisol response that did not return to pre-treatment levels after 180 minutes (Kent *et al.* 1995). The Burdizzo is used for castrating lambs more in Europe than in New Zealand.

Burdizzo castration has been shown to cause a cortisol and behavioural response in 6, 21 and 42 day old calves that were hand reared (Robertson *et al.* 1993) and similarly in 24 week old calves that were isolated from their cow immediately before castration (King *et al.* 1991). However calves 11 weeks old which had also only been removed from their cow just prior to the study showed no cortisol response (King *et al.* 1991). The reasons for the lack of cortisol responses in these cases is not clear. It has been suggested that husbandry practices such as recent isolation of calves may be utilised to reduce the stress of castration (Stafford and Mellor 1993), but this remains to be assessed.

Burdizzo plus Ring: Castration and tailing using this combined method is a relatively new procedure investigated by Kent *et al.* (1993,1995) and Molony *et al.* (1993). The principle behind its use is that the crushing action of the Burdizzo disables the afferent nerves, inhibiting transmission of nociception from the hypoxic nerve endings distal to the crush and the ring. Molony *et al.* (1993) and Kent *et al.* (1993, 1995) found that application of the Burdizzo just distal to the ring, and after its application, caused significantly smaller cortisol and behavioural responses than those exhibited by lambs castrated and tailed with rings. In the original reports (Kent *et al.* 1993; Molony *et al.* 1993) it was unclear whether the crush was a single crush across the entire scrotum or, as is conventional, a crush on each side of the scrotum encompassing a single spermatic cord, which leaves an area of undamaged scrotal tissue between each crush. In the later study by Kent *et al.* (1995) it is clearly stated that the crush was across the entire scrotum.

The distress associated with this method of castration has apparently not been investigated in cattle to date.

Use of local anaesthesia: It has been shown that use of local anaesthesia can abolish the behavioural and cortisol responses seen after castration and tail docking with rubber rings (Wood *et al.* 1991). The local anaesthetic was injected 15 - 20 minutes before treatment into the spermatic cords, scrotum, testes and epidurally into the intervertebral space between coccygeal vertebrae 1 and 2. The duration of anaesthesia in ring castrated lambs is thought to be extended as local anaesthetic in tissues distal to the ring cannot be cleared in the same way as in normally vascularised tissue (Wood *et al.* 1991). In the same way that clearance of local anaesthetic distal to the ring is inhibited, it is likely that distribution of the local anaesthetic within tissues distal to the ring may be inhibited after ring application. Accordingly if sufficient anaesthesia of the area is to be achieved, local anaesthetic may need be injected some time prior to applying a ring. This would make the technique time consuming and not practicable for field conditions. The use of local anaesthetic to prevent or reduce the distress associated with other methods of castration and tail docking had apparently not been investigated at the start of the present work.

1.6.4 Pain (as indicated by distress responses) associated with different methods of castration

Ring: Using plasma cortisol concentrations and behaviour as indicators of distress, which under controlled conditions can probably be assumed to reflect differing intensities of pain, it is possible to speculate on the sources and general changes in nociceptor activity associated with ring castration. It is currently thought that the amount of nociceptor activity (as indicated by pain-induced distress response) after an initial barrage caused by handling of the scrotum and application of the ring, increases to a peak of activity 60 minutes after ring application, and then decreases slowly over the next 1-2 hours in lambs 1 week old (Wood and Molony 1992) and over the next 2-3 hours in lambs 4-5 weeks old (Lester *et al.* 1991b; Kent *et al.* 1993). Ring castration causes ischaemia and eventual death of the testes and the scrotum. It is assumed that extracellular conditions (hypoxia, hypercapnia, hyperkalaemia

and proliferation of inflammatory mediators) in the period from 15 minutes to 3hrs after ring application can cause significant stimulation of nociceptors distal to the ring (Cottrell and Molony, in press). As the testicular tissue becomes more hypoxic, nociceptors may be stimulated in the testis, resulting in increased activity of the afferent spermatic nerve fibres which are still viable for some time after application of the ring (Wood and Molony 1992). Eventually, afferent nerve transmission will be blocked (possibly due to extracellular conditions preventing repolarisation of nerve cells), and the nociceptors themselves will die causing a corresponding decrease in the distress response. This apparently occurs within 2-4 hours of ring application.

A study involving occlusion of the blood supply to rat testes and concurrent recording of activity of the afferent nerves, showed that nociceptor activity in ischaemic rat testes can last for three hours after occlusion of the blood supply (Grubb *et al.* 1990). Nociceptor activity after ring castration, or occlusion of the blood supply, in the scrotum has apparently not been recorded.

At the time the present work began, it was thought by some people that the testes, rather than the scrotum, were the main sources of prolonged nociceptor activity after ring application (Wood *et al.* 1991). This was purportedly supported by a study which involved using local anaesthetic on lambs castrated plus tail docked using rubber rings. However, that argument is contestable on several grounds and will be discussed later in this thesis.

Nociceptor activity is thought to be increased further as hypoxic tissues release substances into the surrounding microenvironment which sensitise and further stimulate the nociceptors (Wood *et al.* 1991). This has been called hyperalgesia, and is a recognised phenomenon occurring after other types of tissue damage (Malmberg and Yaksh, 1992).

The relative contributions of the sources of nociception after ring castration have not been comprehensively researched and hence no conclusive evidence is available.

Burdizzo The initial crush of the burdizzo damages tissues of the scrotum and the spermatic cord. It is presumed that the crushing action on afferent nerve fibres in the scrotum and the spermatic cords causes immediate depolarisation and hence an intense barrage of noxious input to the CNS (Wood and Molony, 1992). However it must be noted that these are only theories and no direct recordings are available to support them. When the burdizzo is applied in the traditional manner (one crush to each spermatic cord and it's associated scrotal tissue), there is an area of undamaged scrotal tissue between the two Burdizzo 'cuts'. This area remains vascularised and innervated. Some of the afferent fibres from the ischaemic scrotal tissue below the burdizzo 'cuts' may however pass through the area of uncrushed tissue. This would allow the transmission of nociception and the hence the experience of pain. Areas of tissue cranial, near, and especially distal to the cuts may have an altered blood supply and hence may become hypoxic and inflamed causing stimulation of nociceptors. (Note: using a burdizzo 'cut' across the entire width of the scrotum is not recommended when the burdizzo is used alone).

Burdizzo plus ring As discussed in section 1.6.3 of this chapter, the crushing action of the Burdizzo is expected to irreversibly damage all nerves (and blood supply) within the spermatic cord and, depending on method of application, the scrotum. This is considered likely to prevent subsequent nociception from ischaemic tissue being transmitted along afferent nerve fibres to the CNS (Kent *et al.* 1993, Molony *et al.* 1993). Hence, the nociception from ischaemic testicular tissue created by the ring (and crushing action of the burdizzo) is unable to be transmitted to the CNS via afferent nerves contained within the spermatic cords.

It is not known what the relative inputs of nociception from the testis or the scrotum are after ring application, therefore the relative importance of damaging the nerves either in the scrotum or the spermatic cords is unknown.

As discussed in section 1.6.3 there are different methods of using a burdizzo. If a burdizzo is applied across the whole width of the scrotum (Kent *et al.* 1995),

it can be expected that most, if not all afferent nerve fibres within the scrotum and the spermatic nerve will be crushed. This may prevent all nociception from any tissue distal to the crush from reaching the CNS. Nociceptors cranial to the crush may be active due to inflammation caused by the crush and also because of a disrupted blood and lymphatic system.

If there are two applications of a burdizzo, each crushing a single spermatic cord and the associated scrotal tissues, it is possible that some afferent neural fibres in the scrotum may remain intact between the two areas of crushed tissue. This may enable nociception from ischaemic scrotal tissue created by a ring to pass through to the CNS.

The prior application of the ring, and application of a burdizzo distal to the ring, means that any substances released from damaged cells are not secreted into the blood stream. This is likely to markedly reduce sensitisation of surrounding tissue or stimulation of the hypothalamic-pituitary-adrenal axis independently of sensory inputs.

1.7 THE THEORY OF NON-SENSORY STIMULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS.

A proposed reason for the prolonged cortisol responses exhibited by lambs castrated with a knife or with a burdizzo was that the hypothalamic-pituitary-adrenal axis may be stimulated independently of sensory input (Kent *et al.* 1995). This may be due to the stimulatory actions of interleukins and other inflammatory mediators on corticotropin releasing factor and ACTH release (Kent *et al.* 1995) (See Fig. 1.4).

If this mechanism contributed significantly to the cortisol response then it would be expected that the use of local anaesthetic, which only acts on the sensory pathways, would not reduce the cortisol response to these treatments. The effects of prior local anaesthetic administration on the cortisol response to surgical castration in lambs has not been documented. However, other studies have demonstrated local anaesthetic abolished the cortisol response to

dehorning in calves for 4 or 8 hours depending on local anaesthetic administration protocol (Petrie *et al.* 1996; C.M. McMeekan, D.J. Mellor, K.J. Stafford, R.A. Bruce, R.N. Ward, unpublished data). This demonstrates that sensory-independent stimulation of the HPA does not occur after dehorning in calves. It would be expected that if sensory-independent stimulation of the HPA occurs after surgical castration in lambs, it would also occur in calves after dehorning. As this is not apparently the case then it is unlikely that sensory-independent stimulation of the HPA plays a significant role in the cortisol response to surgical or burdizzo castration in lambs.

A preliminary report has shown that non-steroidal anti-inflammatory drugs (NSAIDs) and/or local anaesthetic can reduce the cortisol response to Burdizzo castration and tailing (Molony 1993).

There is apparently little evidence to support the theory that the HPA can be stimulated without sensory input, however the possibility can not be discounted.

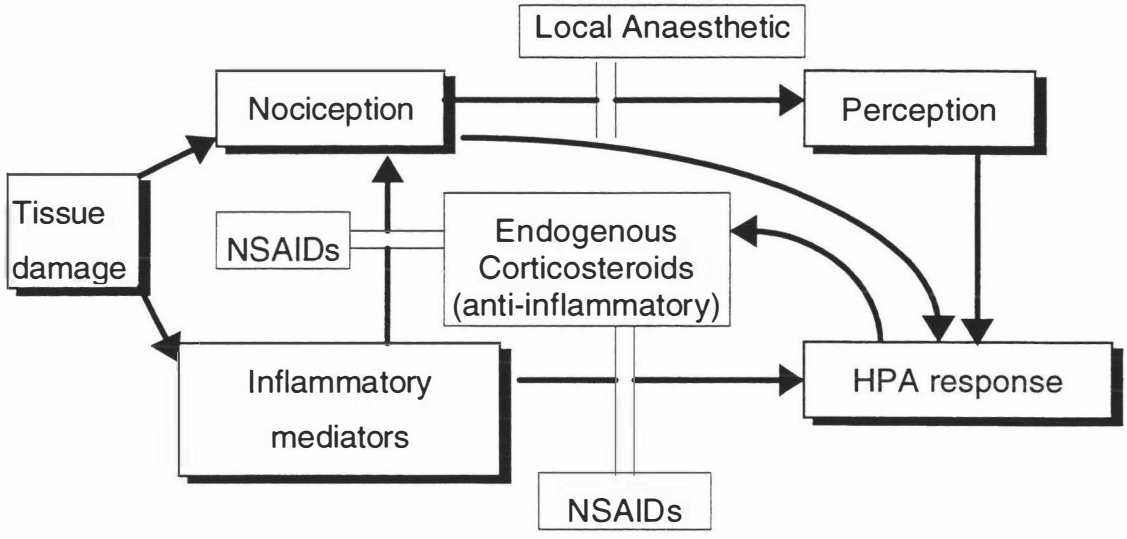


Fig 1.4 The factors that can stimulate the HPA and substances that can inhibit stimulation. NSAIDs = Non-steroidal anti-inflammatory drugs.

|| Indicates that the route of stimulation or interaction is inhibited by the substance.

1.8 AIMS OF THE PRESENT STUDY

The aim of this study was to assess the acute distress associated with castration without tail docking using ring alone, burdizzo, and rings combined with a burdizzo. An attempt was made to answer questions about the relative inputs of nociception from different tissues after castration, by using selective local anaesthetic administration. The practical objectives of this study were to provide recommendations on which castration method is least distressful as well as practicable and to devise efficient and effective local anaesthetic administration strategies that could be recommended to farmers.

The studies in this thesis were designed to answer the following scientific questions:

1. Are there any differences in the physiological (cortisol) or behavioural responses to short scrotum creation or ring, burdizzo, or ring + burdizzo castration?

2. Does the period of burdizzo application to each spermatic cord (1 or 10 seconds) alter the physiological or behavioural stress responses to burdizzo castration?

3. Does the period of burdizzo application (1, 5, or 10 seconds) alter the physiological or behavioural responses when using the burdizzo in combination with a constricting rubber ring?

4. Does the use of different local anaesthetic injection strategies (testes, spermatic cords, scrotum, or spermatic cords + scrotum) alter the physiological and behavioural responses (and consequently provide an insight into the individual sources of noxious input) after ring, burdizzo (10 seconds) or ring + burdizzo (10 seconds) castration?

5. What behaviours are elicited by pain associated with each castration method?

6. Can behavioural responses be used effectively to compare the relative intensity of pain caused by different castration methods?

The studies in this thesis were also designed to address the following practical questions.

1. Which method of castration (ring, burdizzo [1 or 10 seconds], ring + burdizzo [1, 5, 10 seconds]), or short scrotum creation, is the least stressful (as inferred from cortisol and behavioural responses) while still being practicable and can be recommended to farmers?

2. Is there an efficient and practicable method of local anaesthetic administration that can abolish or substantially reduce the stress (as inferred by cortisol and behavioural responses) associated with ring or ring + burdizzo castration that can be recommended to farmers?

CHAPTER 2: LOCAL ANAESTHETIC DISTRIBUTION AFTER INJECTION INTO THREE DIFFERENT SITES.

2.1 CHAPTER SUMMARY

A local anaesthetic/methylene blue dye mixture was used to determine which tissues local anaesthetic infiltrated after different injection strategies and the extent to which the local anaesthetic leaked or diffused from the injection site. The local anaesthetic/dye mixture was injected into the scrotum, spermatic cords, or testes. Although the true extent of diffusion of the local anaesthetic would not have been illustrated by the dye due to the different diffusion properties of the two, it was found that dye injected into the testes leaked out and stained some areas of the scrotum. It was also found that local anaesthetic injected into the scrotum could have anaesthetised the tunica albuginia of the testes. Dye injected into the spermatic cord was not so likely to leak out of them. However dye could have been cleared more quickly from the tissues of the spermatic cords due to the larger number of blood vessels and lymphatic ducts.

2.2 INTRODUCTION

Attempts have been made to alleviate the pain associated with castration in lambs by using local anaesthetic. In a previous study local anaesthetic was administered to the testes, spermatic cord, scrotal neck and tail and successfully alleviated pain associated with ring castration and tail docking (Wood *et al.* 1991). This method is not practical in a farming situation. One of the aims of this thesis was to determine an efficient and effective way of using local anaesthetic to reduce pain associated with castration. Further, it may be possible to determine the relative inputs from the different various sources of

nociception resulting from castration, by injecting local anaesthetic into different sites.

To interpret stress responses after castration with prior local anaesthetic injection, it was necessary to know which tissues would have been anaesthetised.

Neural anatomy

For successful local anaesthesia it is important to have an understanding of the neural anatomy of the target area (Fig. 2.1). The area of skin innervated by the afferent fibres in a peripheral nerve is called the cutaneous area of that nerve (Kirk et al. 1987). Sensory input from the cutaneous area can be blocked either at the level of the afferent fibres or by blocking the main nerve trunk.

The external genitalia of rams receive their nerve supply through three routes (Larson and Kitchell, 1958; Kirk et al. 1987).

1. Ventral branches of the thoracic and lumbar spinal nerves from T9-T12 and L1,2 (cranial and caudal iliohypogastric nerves) (Fig. 2.1)

2. Genital femoral nerve (also known as the inguinal or external spermatic nerve) made up of branches of the lumbar spinal nerves L2-L4; and

3. Ventral branches of the sacral spinal nerves S1-S4 from which the pudendal nerve originates and then branches into the scrotal nerve and the deep perineal branch (DNP).

Afferent fibres from the testes in rams are contained within the superior spermatic nerves (Cottrell and Molony, in press) which are enclosed within the spermatic cords. Afferent fibres of the spermatic cords can reach the spinal column by way of the hypogastric nerve and mesenteric plexus which connect to the splanchnic nerves and thence to the dorsal roots of the spinal column (Setchell and Brooks 1988). Fibres also connect to the sacral spinal column via the sacral nerves (S1-S3) (Figure 2.1).

The scrotum is also innervated by the distal cutaneous branch of the sacral plexus, and the scrotal nerve (Kirk *et al.* 1987; Lester 1991).

Nociceptor location.

The location of nociceptors in the testes, scrotum and related tissues apparently has not been researched extensively. In the dog, polymodal receptors, that can function as nociceptors, have their receptive sites close to the surface of the tunica vaginalis visceralis which surrounds the testes and the epididymis (Kumazawa and Mizumura 1980a). Cottrell and Molony (In press) have found that there were some nociceptors present within the pampiniform plexus in lambs.

Although the scrotum contains thermal receptors involved in maintaining scrotal temperature (Schingnitz 1981), the presence of nociceptors in the scrotum, which is likely, does not appear to have been documented.

Anatomy of Scrotal and Testicular Tissues

As well as understanding the neural anatomy of the area it is important to be familiar with the general anatomy of the tissues into which local anaesthetic is to be injected.

The wall of the scrotum is made up of four layers, the most superficial being the skin. Beneath this is the tunica dartos which is a fibrous and muscular layer. The tunics of each side meet centrally to form a median septum (scrotal septum) which divides the pouch into two cavities. Beneath the tunica dartos is the ill defined scrotal fascia, and beneath this is the tunica vaginalis. Along the caudal wall of each cavity, the tunica vaginalis is reflected to cover the testis and the spermatic cord, so that the part lining the cavity is called the parietal layer, or the tunica vaginalis communis, and the reflected portion the visceral layer, or tunica vaginalis propria.

The testicular tissue consisting of seminiferous tubules, sertoli cells, etc is contained within a layer of tissue called the tunica albuginea. This maintains the testis under mild pressure.

The anatomy of the scrotal and testicular tissues of the ram is shown in Fig. 2.2.

Aims of the present study

The main aim of the present study was to determine how accurate the different injection techniques were. Further, it was hoped that the extent to which the anaesthetic diffuses during the 15 minutes between injection and castration would be indicated by the diffusion of dye.

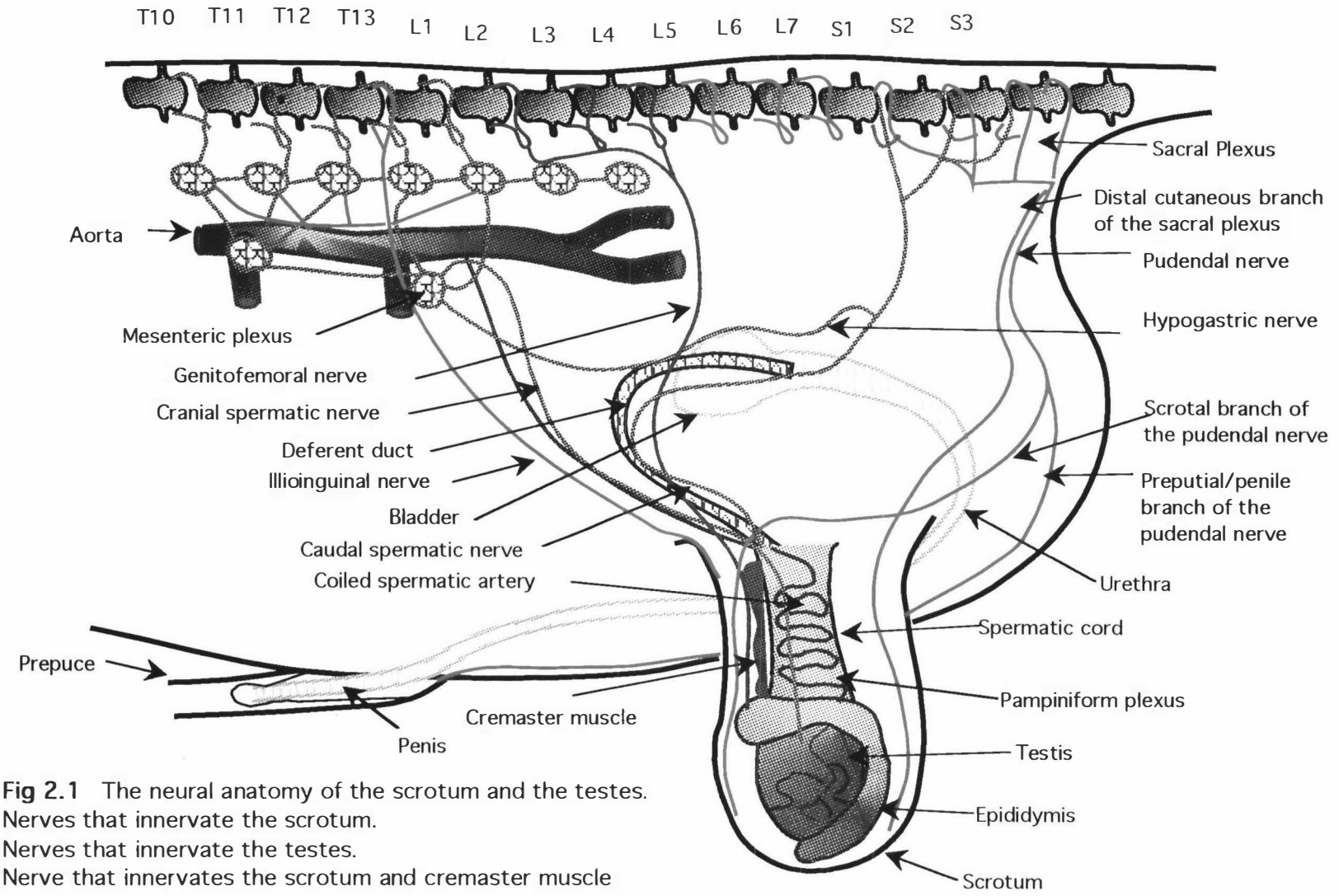


Fig 2.1 The neural anatomy of the scrotum and the testes.
Nerves that innervate the scrotum.
Nerves that innervate the testes.
Nerve that innervates the scrotum and cremaster muscle

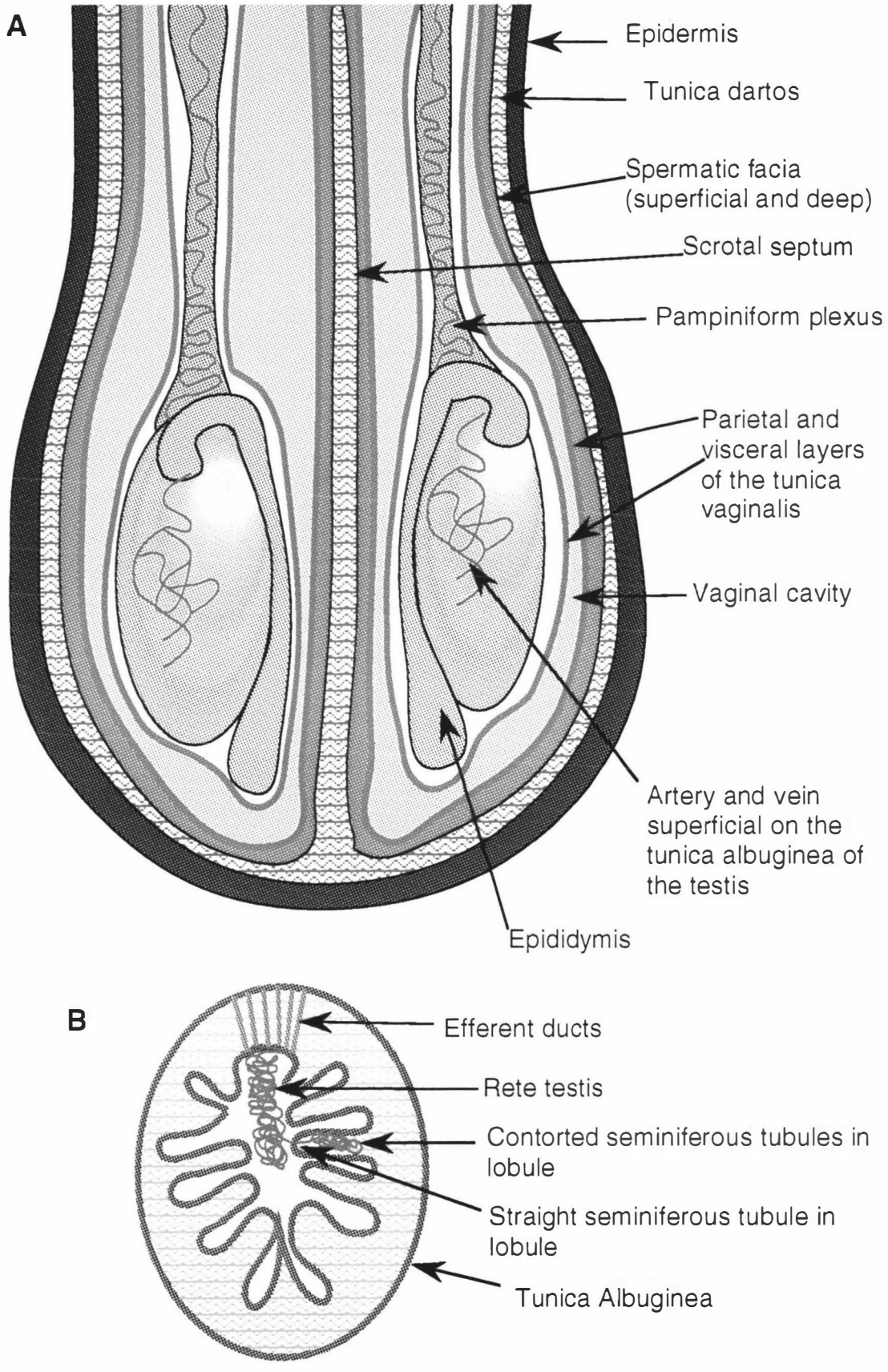


Fig 2.2 A) Tissue structure of the scrotum and it's contents. **B)** Cross section of testis. N.B. tissue structures not to scale.

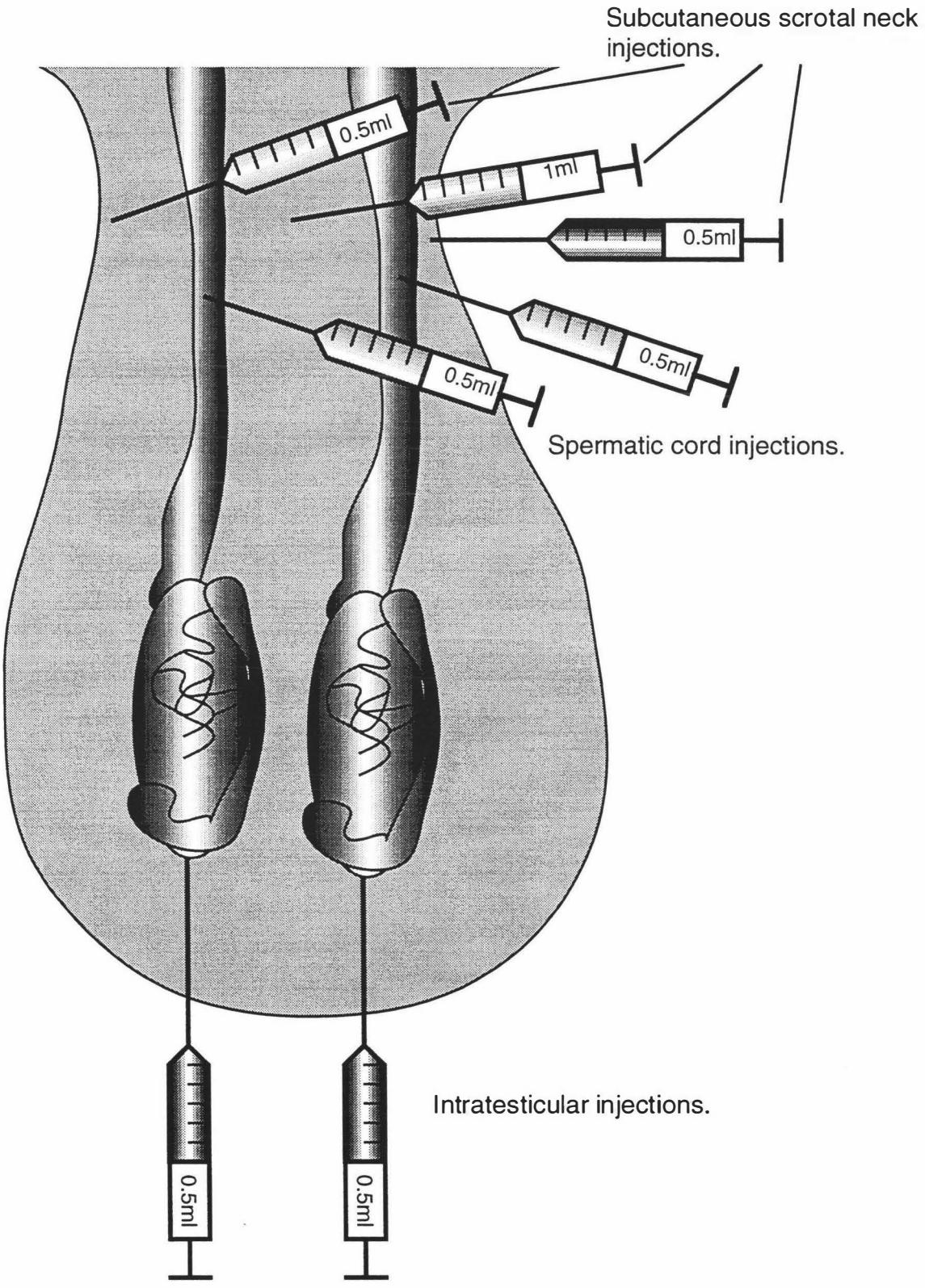


Fig. 2.3 The sites and amounts of local anaesthetic injected in the present study.

2.3 MATERIALS AND METHODS

2.3.1 Animals

Twelve Coopworth/Romney cross ram lambs were used in this study. They were divided into three groups with four lambs in each group. Each testis was treated separately hence each group had a potential for 8 treatments. The lambs were injected with local anaesthetic mixed with methylene blue dye using a 21 gauge needle. The mixture consisted of 0.5% methylene blue dye (3 parts) and lignocaine (Nopaine, Ethical agents Ltd, Auckland) local anaesthetic solution (4 parts). Fifteen minutes after injection the lambs were killed by injecting sodium pentobarbitone ("Lethobarb") intravenously. Immediately after death the scrotal contents were dissected and the extent of dye infiltration recorded.

2.3.2 Treatments

Intratesticular Injection: The local anaesthetic/dye mixture (0.5ml) was injected into the testis at or near the testicular pole (Fig 2.3).

Scrotal Neck Injection: The local anaesthetic/dye mixture (1 ml) was injected into the anterior surface of the scrotal neck and into either side of the scrotal neck (0.5ml each side) (Fig. 2.3).

Spermatic Cord Injection: The local anaesthetic/dye mixture (0.5ml) was injected into each spermatic cord through the scrotal neck (Fig. 2.3).

2.3.4 Presentation of results

The results are presented in Table 2.1 which shows the percentage of lambs injected in a particular site showing evidence of the dye in different areas. Each side of the lamb's scrotal contents were considered separately, as they are separated by an invagination of the tunica dartos called the scrotal septum (Figure 2.2).

One lamb in the intratesticular group and one lamb in the spermatic cord group had only one side injected with the anaesthetic/dye mixture. This was to provide control tissue for observation.

2.4 RESULTS

Intratesticular Injection: Lambs injected in the testis exhibited localised evidence of dye within the subcutaneous fascia around the injection site. There were obvious "needle tracks" through the fascia and tunica albuginea where the dye had leaked out of the testis (Fig. 2.4).

The injection site was usually at the caudal pole which is close to the proper ligament of the testis. The proper ligament showed evidence of stain in each testis injected (Fig. 2.4). In one instance there was evidence of a free blood clot within the cavity of the scrotum which was likely to represent blood which had leaked from the puncture of a blood vessel in the tunic.

Spermatic Cord Injection: Lambs injected in the spermatic cord varied in the amount of dye evident in different areas (Fig. 2.5). Some lambs exhibited slight leakage from the injection site but others did not. One injection, which resulted in a significant amount of dye within the scrotal fascia, was suspected to have passed subcutaneously rather than into the spermatic cord. No dye was evident in the epididymis, however natural pigmentation was present in some cases.

Dye was evident in all spermatic cords into which it was injected, diffusing longitudinally approximately one centimetre in both directions from the injection site. Stereo microscope observation suggests that dye may not always infiltrate all layers of tissue within the spermatic cord. For example, one lamb showed

evidence of dye surrounding the pampiniform plexus but not entering it. The pampiniform plexus was however, penetrated in one lamb as one blood vessel on the side of a testis stained blue. This is thought to have happened because of infiltration of the pampiniform plexus and possible puncture of the spermatic artery by the needle.

The tunica albuginea exhibited evidence of anaesthetic/dye infiltration in a small area at the site of injection in most cases.

Scrotal Neck Injection. In all cases there was a large concentration of dye within the scrotal fascia at the level of the injection site (Fig. 2.6). The dye diffused radially from the injection site for a distance of approximately 1 cm. The epididymis was stained in one lamb, however the matter was again confused by the presence of natural pigmentation. The surface of the tunica albuginea around the testis was only stained in one instance, however, at the level of the spermatic cord the tunica albuginea was consistently stained.

Natural Pigmentation

Natural pigmentation of the epididymis in some lambs confused initial observations but histological preparation later revealed concentrations of melanocytes within the epididymis that when clumped together looked similar to, but distinguishable from dye markings (Fig. 2.7).

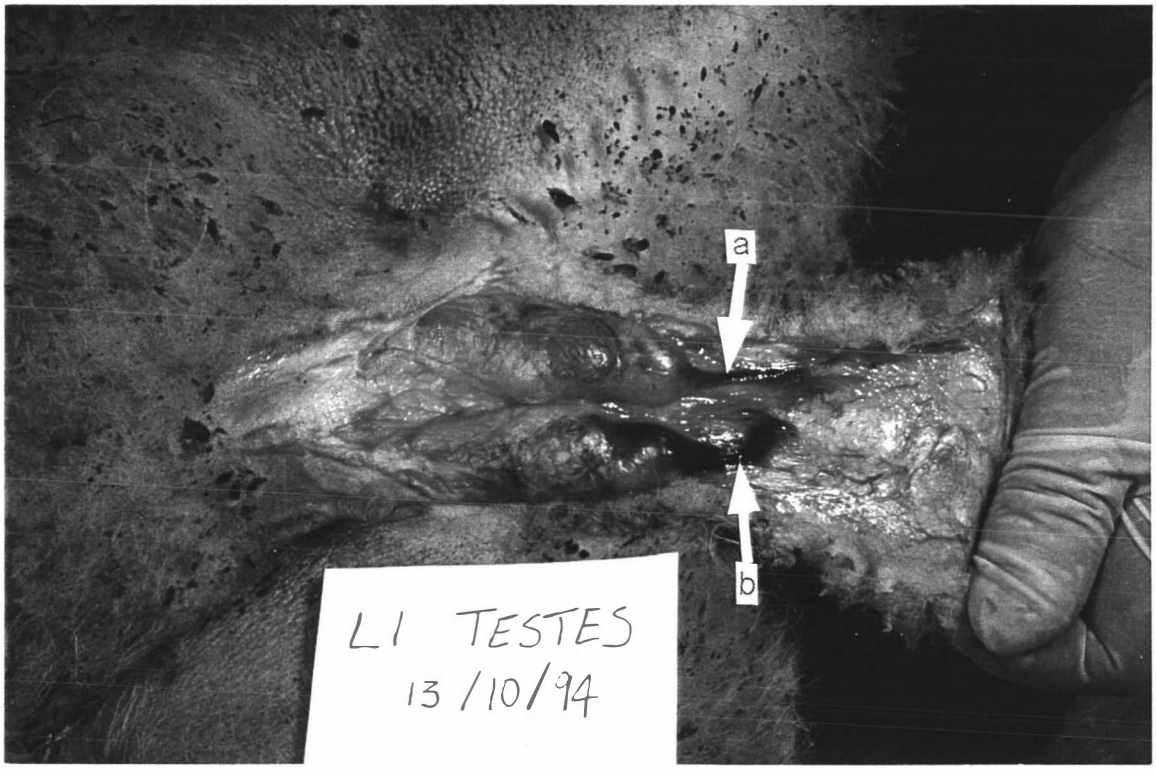


Fig. 2.4 Evidence of dye leakage from intratesticular injection of the local anaesthetic/dye mixture. Arrows indicate; a) dye leaking out of the testes through needle track, b) the presence of a haematoma within needle track.

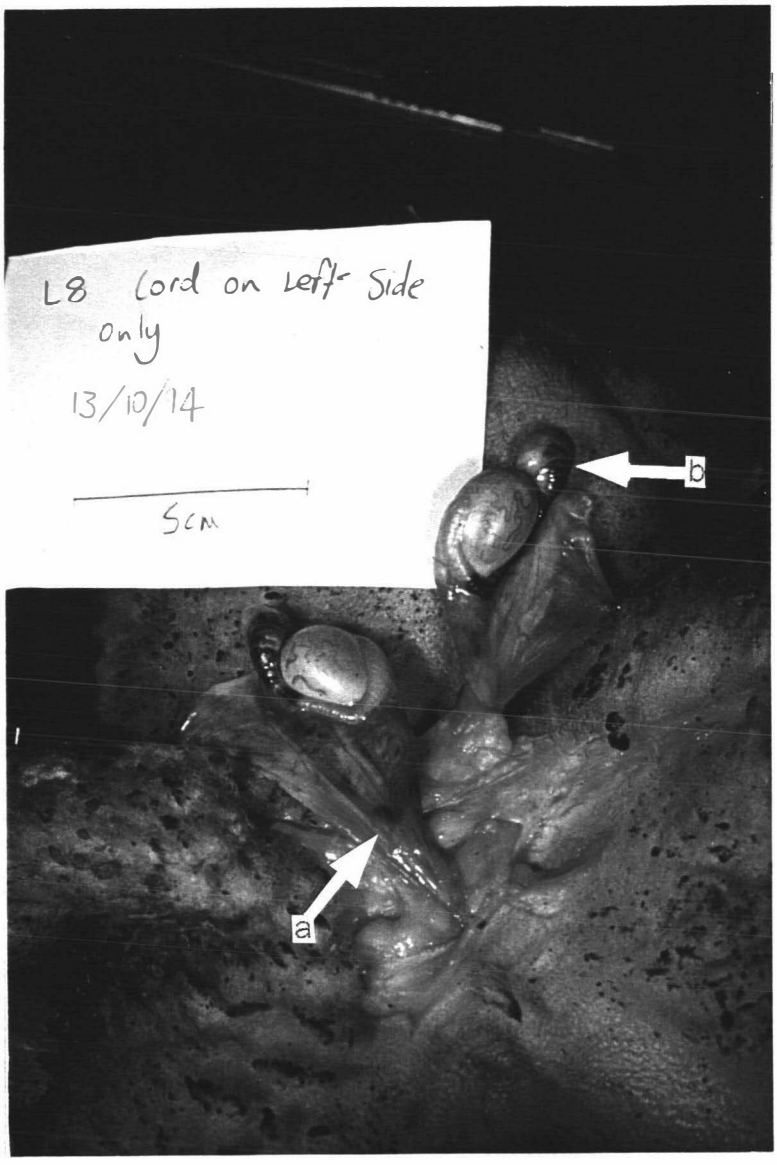


Fig. 2.5 Evidence of dye present within spermatic cord after injection of anaesthetic/dye mixture into spermatic cords(a). Note that the dark area of the epididymis (b) was confirmed as natural pigmentation resulting from melanocytes, see Figure 5.6.

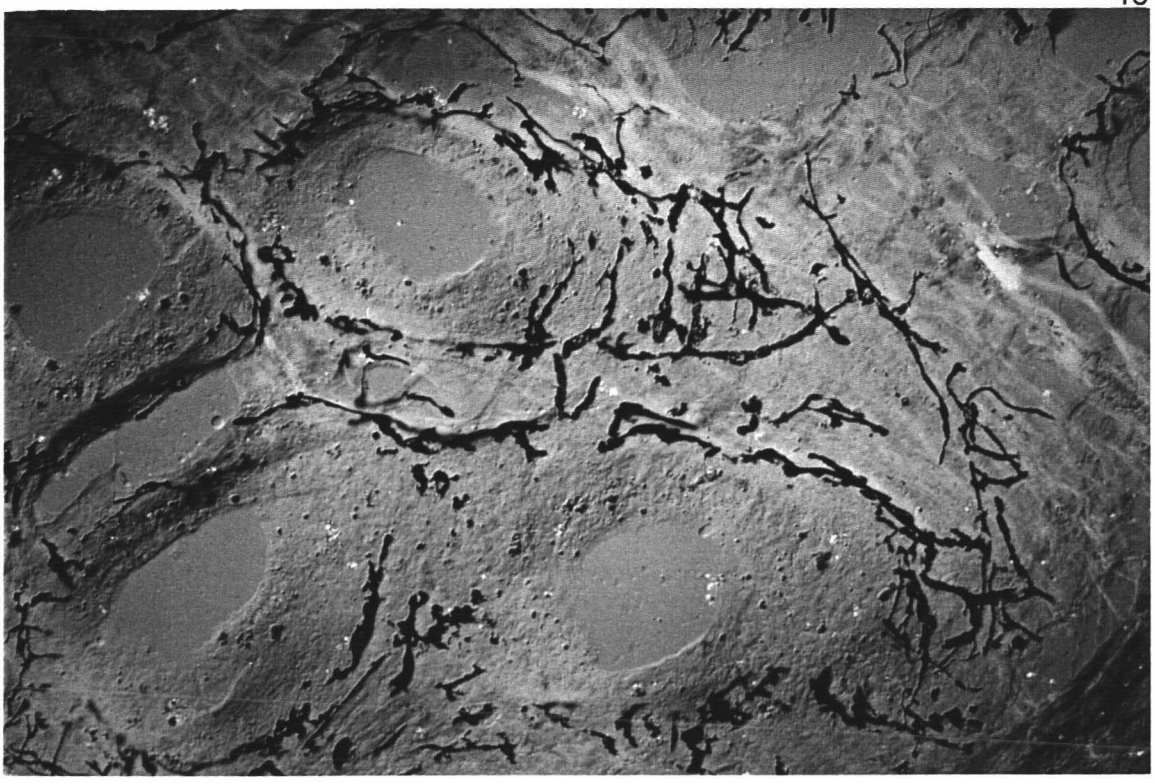


Fig. 2.6 Cross section of the epididymis revealing presence of melanocytes rather than dye. (20X magnification.)

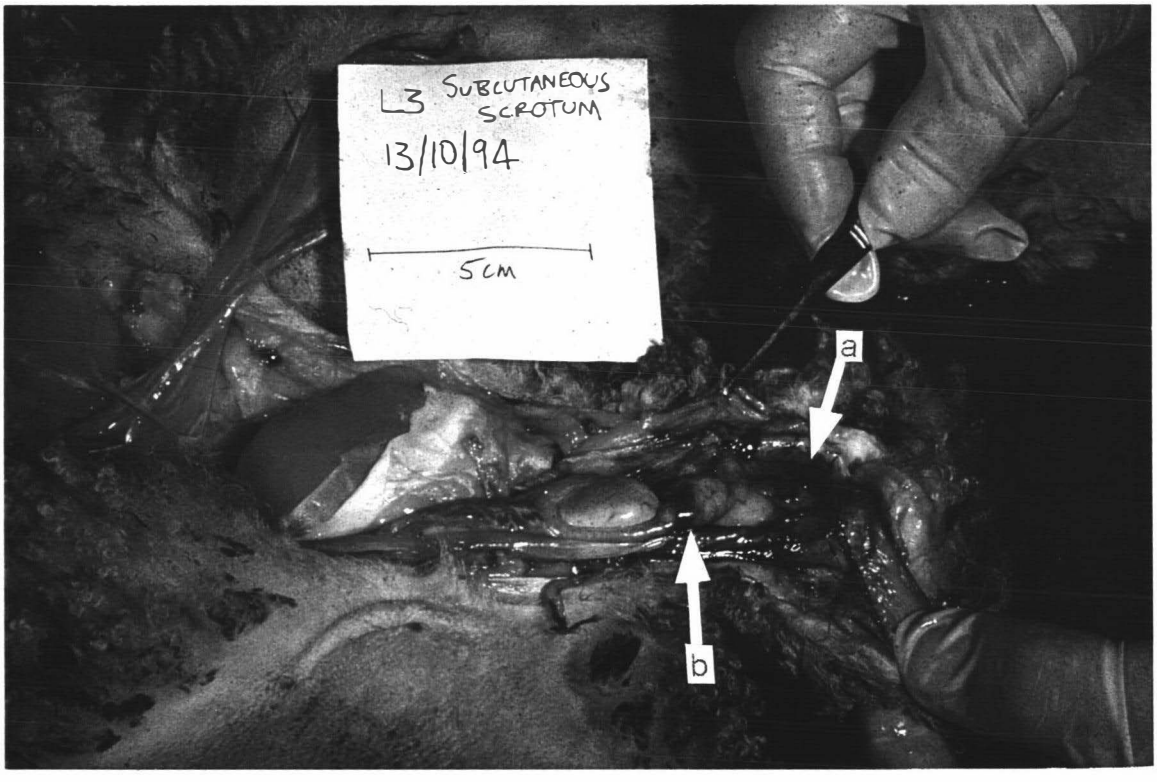


Fig. 2.7 Evidence of dye present after subcutaneous injection into the neck of the scrotum (a). Note the presence of dye on the surface of the epididymis (b).

Tissue	Subcutaneous Facia	Proper Ligament	Leakage from needle site	Lymph Nodes	Within Epididymis	Within Testis	Tunica vaginalis		Within spermatic cord
Injection site							testis	cord	
testis	0%	100%	100%	0%	0%	100%	86%	29%	29%
Spermatic cord	43%	0%	71%	14%	0%	14%	0%	86%	71%
Scrotal neck	100%	0%	100%*	14%	14%	0%	100%	100%	

Table 2.1 The percentage of lambs in each treatment group that showed evidence of dye in a particular tissue. NB Each side of the lamb's scrotum and contents were considered separately. Number of sides studied in each group = 7, except for scrotal injection which = 8.

* Due to the nature of this injection, leakage of the dye from the site is expected.

2.5 DISCUSSION

There is a major limitation of this study that must be taken into consideration before analysing the results. Methylene blue dye may not diffuse as effectively as lignocaine through tissue. Lignocaine is very soluble in water so will rapidly diffuse through tissue and also will be quickly absorbed into the circulatory system (Ritchie and Greene, 1990). The diffusion rate of methylene blue dye is likely to be different to that of local anaesthetic and therefore the methylene dye may not have been a good indicator of the extent of diffusion of local anaesthetic. However the dye indicated where the local anaesthetic was actually injected and if leakage of local anaesthetic from the injection site had occurred.

Intratesticular injection of local anaesthetic could anaesthetise parts of the scrotum.

The results from this study indicate that when local anaesthetic is injected into the testis it is likely to leak back out of the needle puncture and diffuse onto the surrounding scrotal fascia and tunica dartos. This may anaesthetise the lower part of the scrotum as well as the testis. Local anaesthetic probably leaked out of the testes because they are contained within a tough tunica which maintains the testicular tissue under pressure. As noted above the true extent of local anaesthetic diffusion may not have been demonstrated by the presence of methylene blue dye. Dye diffusion could indicate the minimum diffusion of local anaesthetic, but on the other hand the diffusion of dye may be more extreme than local anaesthetic.

The scrotal injection may also anaesthetise the surface of the testes.

Due to the amount of local anaesthetic injected into the scrotal neck some may also anaesthetise the surface of the testes. It has been demonstrated that the polymodal receptors are predominantly situated close to the surface of the testes within the tunica vaginalis visceralis (Kumazawa and Mizumura 1980a). Therefore it seems likely that if the surface of the testes came into contact with the large amount of local anaesthetic injected into the scrotum, then anaesthesia of the testicular polymodal receptors may occur. Indeed, in one instance in the current study, evidence of dye was seen within the tunica albuginia of the testes. The scrotal neck injection is likely to prevent nociception from the cutaneous areas of the scrotum innervated by the scrotal branch of the pudendal nerve and the distal cutaneous branch of the sacral plexus.

It is also possible that local anaesthetic injected subcutaneously into the scrotum could diffuse into the spermatic cord and block the spermatic nerves. This would effectively prevent transmission of nociception from the testes as well as the scrotum.

Injection of local anaesthetic into the spermatic cord is likely to have successfully prevented activity of afferent nerve fibres within the spermatic nerve.

The spermatic cord injection was shown to penetrate the spermatic cord in all cases in this experiment. This allayed fears that this injection may not always be successful due to the difficulty in identifying the exact tissue penetrated. The superior spermatic nerve was likely to have been blocked by this injection, preventing activity of afferent nerves from testicular nociceptors. It seems unlikely, unless leakage occurred, that the genitofemoral nerve will have been anaesthetised by this injection. However, the extent to which the genitofemoral nerve is involved in the transmission of nociception from the testes or scrotum apparently has not been documented.

If dye seemingly represents the extent of local anaesthetic diffusion then the results indicate that local anaesthetic does not leak out of the spermatic cord

and anaesthetise any tissues of the scrotum. If the true extent of local anaesthetic movement is not indicated by the dye then the possibility that some tissues of the scrotum were also anaesthetised cannot be ruled out. However, the cortisol responses of lambs described in Chapter 3 may help to clarify this matter.

It may be anticipated from the results of this study:

- That local anaesthetic when injected into the spermatic cords will only block the nerves contained within the spermatic cord and hence prevent transmission of nociception from testicular tissue.

- That local anaesthetic when injected into the scrotum will anaesthetise the scrotum and testes and hence prevent any nociception from both tissues.

- That local anaesthetic injected into the testes may leak out and anaesthetise parts or all of the scrotum hence preventing or substantially reducing nociception from the scrotum and testes.

The following implications on castration effects may be anticipated from the results of this study.

- That application of a rubber ring to the neck of the scrotum will prevent any uptake of local anaesthetic distal to the ring hence prolonging the effect of the local.

- That the application of the burdizzo will also disrupt the blood supply of the spermatic cords and parts of the scrotum and so will also prolong the effects of local anaesthetic within the spermatic cord distal to the crush.

- Local anaesthetic present in the spermatic cord will be absorbed much more quickly into the systemic circulation and lymphatic system due to the presence of the pampiniform plexus of venules, arterioles and lymphatics.

CHAPTER 3: EFFECTS ON PLASMA CORTISOL CONCENTRATIONS OF CASTRATING LAMBS USING DIFFERENT METHODS, WITH OR WITHOUT PRIOR LOCAL ANAESTHETIC ADMINISTRATION .

3.1 CHAPTER SUMMARY

The pain-induced distress caused by different methods of castrating lambs was assessed using plasma cortisol concentrations. Different local anaesthetic strategies were also used to reduce or abolish the cortisol responses and therefore presumably pain-induced distress. The castration methods assessed were ring, short scrotum, burdizzo plus ring, and burdizzo. The burdizzo was used in combination with the ring on the hypothesis that the burdizzo would sufficiently damage the afferent nerves from the testes to prevent transmission of nociception caused by ring application. However, the burdizzo used in the conventional manner (one application to each spermatic cord and the surrounding scrotal tissue with no overlap of the 'cuts') was not successful in reducing the cortisol response caused by ring application. This was thought to be due to the scrotum not being crushed across its whole width. The duration of burdizzo application (1, 5, or 10 seconds) had no significant effect on the cortisol responses to burdizzo plus ring castration.

The burdizzo used alone to castrate lambs was found to cause a more prolonged cortisol response than ring or burdizzo plus ring castration. The duration of burdizzo application (1 or 10 seconds) was found to have no significant effect on the cortisol response to burdizzo castration.

Short scrotum creation caused less distress than ring application (as indicated by plasma cortisol results).

Local anaesthetic injected into the scrotum, spermatic cords plus scrotum, or testes virtually abolished the cortisol response to ring or burdizzo plus ring

castration. However local anaesthetic injected into the spermatic cord did not significantly reduce the cortisol responses to the same treatments. Local anaesthetic injected into the spermatic cords or the scrotum had no effect on the cortisol response to burdizzo castration. Although incomplete anaesthesia is the most likely explanation for this result, sensory independent stimulation of the hypothalamic-pituitary-adrenal axis cannot be ruled out.

The effects of selective local anaesthetic administration on the cortisol response to castration along with the cortisol response to short scrotum creation indicated that both the scrotum and testes were sources of nociception after ring castration.

3.2 INTRODUCTION

In New Zealand many farm systems require the castration of lambs for management reasons as outlined in Chapter 1. However, there is a growing interest in the assessment and alleviation of the distress associated with castration and other animal husbandry practises. This reflects the growing concern for the well-being of animals that are under human care. In this chapter, plasma cortisol responses, which can be used as indicators of distress, are monitored in lambs following castration.

There are limitations to the use of plasma cortisol concentrations as an indicator of distress as discussed in Chapter 1 (Rushen 1986). However, under carefully controlled conditions, plasma cortisol concentrations have been shown to be a useful indicator of the magnitude and duration of distress experienced by lambs and other animals (Mellor and Murray, 1989a; Stafford and Mellor, 1993). When two or more different parameters (eg. behaviour and plasma cortisol responses) are used together, a more comprehensive representation of the duration and magnitude of the distress experienced may be determined. Plasma cortisol concentrations have been used to assess the acute distress experienced by lambs after castration with or without tail docking (Shutt *et al.* 1988; Mellor and Murray, 1989a,b; Lester *et al.* 1991a,b; Kent *et al.*

1993,1995; Molony *et al.*1993) and calves after castration (Mellor *et al.* 1991; Robertson *et al.* 1994).

The plasma cortisol response to castration and tailing in lambs is thought to be caused by nociception from damaged tissue resulting in pain and consequently distress. There is however an unsubstantiated proposition that inflammatory mediators released from wounds, as well as stimulating nociceptors, may stimulated the HPA independently of sensory pathways (Kent *et al.* 1993, 1995). If this does occur, then it would be expected that the prior administration of local anaesthetic to the area of tissue damage would be ineffective in abolishing the plasma cortisol response because local anaesthetic would only prevent nociception and not the production of inflammatory mediators. Local anaesthetic administered to the cornual nerve prior to dehorning abolished the cortisol response to dehorning in calves for 4 hours (Petrie *et al.* 1996b) and for up to eight hours (C. McMeekan, D.J. Mellor, K.J. Stafford, R.A. Bruce, R.N. Ward, unpublished data). Dehorning in calves would be expected to produce similar inflammatory mediators as surgical castration in lambs and hence the proposition of sensory-independent stimulation of the HPA seems unlikely.

Sensory-independent stimulation of the HPA is unlikely to play a major part in the cortisol response seen in lambs castrated with a rubber ring. This is because access of chemical mediators, from damaged or dying tissue, to the circulatory system is prevented by the rubber ring constricting the venous drainage from tissues distal to it. Furthermore, it has been shown that local anaesthetic administered into the scrotum, spermatic cord, testes and tail before ring application, virtually abolished the plasma cortisol response normally seen after castration and tailing with rubber rings (Wood *et al.* 1991). This would not be expected if stimulation of the HPA occurred independently of the nociceptors and their afferent nerves which are blocked by local anaesthetic.

The distress caused by different castration methods, as determined by plasma cortisol responses, is discussed below.

Surgery.

With surgical castration, as it is often conducted on farms, the lower part of the scrotum is removed using a sharp knife and the testes are pulled out, breaking the spermatic cord usually within the inguinal canal. This causes a marked plasma cortisol response that lasts for up to eight hours after treatment (Lester *et al.* 1991a,b; Kent *et al.* 1993). It is likely that the prolonged and marked cortisol response is due to noxious sensory input (probably nociception) from the damaged scrotal and spermatic cord tissue.

Although it is not absolutely certain that noxious sensory input is the primary cause of the large and prolonged cortisol response to surgical castration in lambs it is very probable. Therefore it is better to err on the side of caution and hence this method is not recommended as a means of castration or tailing lambs (Stafford and Mellor, 1993). For this reason, surgical castration was not further investigated in this study.

Ring Castration and Short Scrotum.

In ring castration a constricting rubber ring is applied to the neck of the scrotum with the testes distal to the ring. This causes ischaemia and the eventual sloughing of the testes and scrotum. The short scrotum technique involves placing a constricting rubber ring onto the scrotum, distal to the testes. The testes are pushed up against the abdominal wall, raising their temperature and rendering the lamb infertile (Probert and Davies, 1986).

Ring castration caused a marked rise in plasma cortisol concentrations thus indicating significant distress. However the magnitude of the rise was lower than that exhibited by surgically castrated lambs, and the cortisol response was completed by three hours after treatment (Mellor and Murray, 1989 a, b; Lester *et al.* 1991a; Mellor *et al.* 1991; Kent *et al.* 1993; Kent *et al.* 1995).

In ring castration the cortisol response is probably caused by noxious sensory input (nociception) from the ischaemic tissues distal to the ring. It is not known

what relative contributions noxious inputs from scrotal or testicular tissue make to the total cortisol response to ring castration. Lester *et al.* (1991) showed that short scrotum + tailed lambs had a similar cortisol response to ring castrated + tailed lambs. This suggests that of the three possible sources of noxious stimulation (scrotum, testes, tail) the scrotum plays a large part in nociceptor input, but to clarify this further, the ring castration and short scrotum techniques need to be performed alone to avoid the complicating influences of tailing.

Selective local anaesthetic administration may determine the sources of nociception after ring castration and the relative contributions of different tissues to the total noxious input caused by ring castration. By selectively blocking the nociceptor activity in different tissues such as those of the scrotum or the testes, and then measuring the distress after ring castration, it may be possible to determine which tissue, either scrotal or testicular, is the major source of nociception.

This same strategy may also indicate an economical and efficient way of local anaesthetic administration that effectively reduces or abolishes the distress caused by ring castration.

Burdizzo castration.

The burdizzo or bloodless emasculator is a clamp-like device which is used to crush the scrotum and the underlying spermatic cords, irreparably damaging the spermatic nerve and the blood supply to the tissues distal to each crush. Used in the traditional manner, the burdizzo is applied once or twice for 5-10 seconds to each spermatic cord and its associated scrotal tissue. This causes ischaemia and atrophy of the testes and ischaemia to parts of the scrotum. Usually enough scrotal tissue with undamaged vascularisation is left between the two 'cuts' to prevent atrophy of the scrotum.

The cortisol response to traditional burdizzo castration alone had not been assessed when this study had been planned, however Kent *et al.* (1995) have assessed the cortisol response to burdizzo castration combined with tailing.

Tailing using the burdizzo involves one crush across the width of the tail with no effort being made to place the burdizzo between two vertebrae. Burdizzo castration and tailing elicited an immediate and prolonged plasma cortisol response that was as great in magnitude and longer in duration (at least 180 minutes) than that seen in lambs castrated and tailed with rings (Kent *et al.* 1995). The prolonged response was thought to have been caused by inflammatory factors that, as well as stimulating nociceptors surrounding the crushed tissue, may have caused sensory-independent stimulation of the HPA (Kent *et al.* 1995). Use of local anaesthetic or non-steroidal anti-inflammatory drugs (NSAIDs) before burdizzo castration and tailing has been shown to significantly reduce the magnitude of the cortisol response (Molony 1993). When used together NSAIDs and local anaesthetic abolished the cortisol response to burdizzo castration altogether (Molony 1993). At the time the present study was planned and carried out there was no report on the cortisol response to burdizzo castration without tail docking, therefore the magnitude and duration of the cortisol response needed to be defined.

Burdizzo and ring combination.

In burdizzo plus ring castration, the rubber ring is placed and then the burdizzo can be applied in two different ways. The burdizzo may be applied on two separate occasions crushing each spermatic cord and the associated scrotal tissue or once across the entire width of the scrotum.

The concept of using the burdizzo in combination with the ring resulted from the hypothesis that disabling the afferent nerves from the testes by crushing them with the burdizzo, would decrease nociceptor input from testicular tissue (Molony *et al.* 1993, Kent *et al.*1993). This idea remained untested at the time the study described in this chapter was planned and performed. The hypothesis was formulated after the observation that neural activity in ischaemic rat testicular tissue can continue for up to three hours after occlusion of the blood supply (Grubb *et al.* 1990). It has recently been shown that in lambs, it is likely that afferent activity in unmyelinated fibres associated with nociceptors can continue for upwards of 90 minutes after application of a

rubber ring (Cottrell and Molony, in press). However, the relative importance of nociception from the scrotum and the testes has still to be established so the importance of disabling afferent nerve fibres from the testes remains unclear.

The burdizzo castrates by crushing the spermatic cords and the associated scrotal tissue. When used in combination with the ring, the burdizzo crushes, and thereby prevents afferent nerve transmission of nociception from ischaemic tissue created by the ring. Thus, apart from the initial barrage caused by the crushing action of the burdizzo, no transmission from the nociceptors distal to the crush should reach the central nervous system (Kent *et al.* 1993; Molony *et al.* 1993). The studies by Kent *et al.* (1993) and Molony *et al.* (1993) have shown that in some lambs using the burdizzo on the scrotum and tail after ring application does reduce the plasma cortisol response to ring castration and tailing. It was unclear in the description of these studies exactly how the burdizzo was applied. There was a large amount of variation in cortisol responses between lambs in these trials suggesting that the technique was more successful on some lambs than others. This suggested that the method needed further evaluation. A further study (Kent *et al.* 1995) has shown that the use of the burdizzo across the tail and the entire width of the scrotum after ring application caused cortisol responses significantly lower than those seen in lambs castrated and tail docked with the ring alone. The use of the burdizzo on the tail may be a confusing influence on the cortisol response exhibited by these lambs. As no effort was made to apply the burdizzo between two vertebra, it seems likely that in some cases bone may have crushed, which may caused differing levels of noxious input compared to lambs that bone was not crushed. Lester *et al.* (1991a) have shown tailing with a ring is relatively benign and hence the use of the burdizzo on the tail is probably unnecessary.

Local anaesthesia and systemic analgesia.

Local anaesthesia is used to block sensory input from damaged tissues. The cortisol response to ring castration and tailing can be abolished by injecting local anaesthetic into the scrotum, testes, spermatic cord, and into the tail (Wood *et al.* 1991). There are a number of reasons why the use of local

anaesthetic in large scale farming situations is not common. It is considered to be expensive, time consuming and complicated and can cause sepsis. It is also considered to be unnecessary by some farmers. For these reasons, seeking efficient and simple methods of local anaesthetic administration is warranted. It may be that a significant reduction but not total elimination of the cortisol response (indicating the distress experienced) to castration is an acceptable objective (Stafford and Mellor 1993), as long as the method of administration is feasible under normal farming conditions.

The present study was designed to address the following questions.

1. Are there any differences between the cortisol responses in lambs castrated using ring, burdizzo for 1 or 10 seconds, ring + burdizzo for 1, 5, or 10 seconds, or short scrotum techniques?

2. Does the injection of local anaesthetic into different sites (testes, scrotum, spermatic cords, or scrotum and spermatic cords), and thereby selectively reducing sensory input from different areas, influence cortisol responses to castration using the ring, burdizzo (10 seconds), or ring + burdizzo (10 seconds) methods?

3. What are the predominant sources (scrotum, testes) of noxious sensory input which stimulate the cortisol response after ring, burdizzo, or burdizzo + ring castration?

4. What inferences about distress can be made from the cortisol responses exhibited by the lambs castrated with the different methods assessed in this study and can these lead to practical advice for farmers in order to reduce the distress experienced by lambs?

3.3 MATERIALS AND METHODS

3.3.1 Animals

One hundred and seventy four Romney ram lambs with a mean age of 43 days (range 28-64) and a mean weight of 15.3 kg (range 8 kg - 26.8 kg) were used for this study. 14 lambs that were initially used in control groups in the first few days of the study, were used again at least two weeks later to make up numbers in other groups. Mellor and Murray (1989b) have shown that prior stimulation of the HPA does not affect the magnitudes or durations of subsequent cortisol responses. The cortisol responses from these lambs when castrated were within the range of the responses from the other lambs in the same group. Lambs castrated in this study were scheduled for castration according to the usual husbandry practise on Massey University Keebles Farm.

After the ram lambs had been weighed, and their necks clipped to facilitate jugular venipuncture, they were penned together with their ewes (pen size 1.5 m by 2 m) at 5 pm the night before the study. Each lamb was marked with a scourable marker spray to allow easy identification for subsequent bleeding and behavioural observations. If a ram lamb had a ewe co-twin, they were both held in the same pen. The lambs (including any ewe lamb twins) were separated from their dams approximately 1 hr before the study by a wire mesh fence allowing them to see, smell, and hear their dams but not to feed (Fig. 3.1).

Fig. 3.1 Pen arrangement to separate lambs from their mothers.

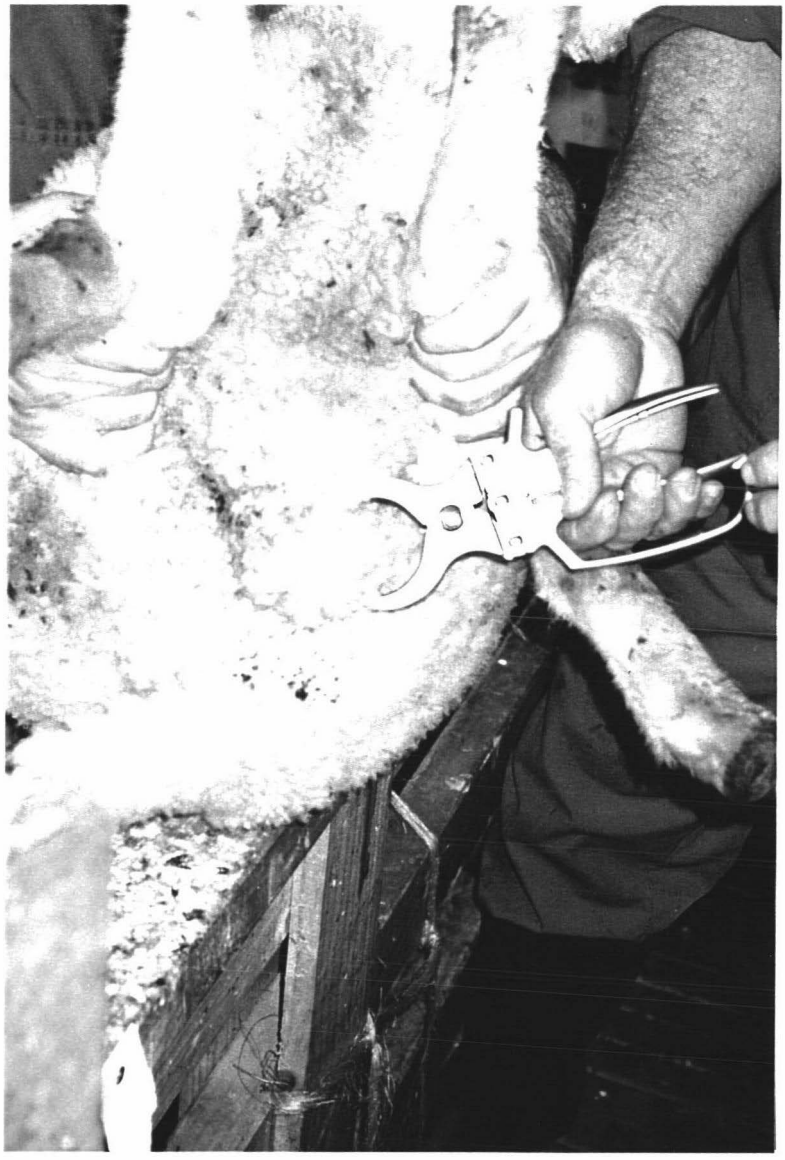


Fig. 3.2 Method of holding lambs for treatment, in this case burdizzo castration.

3.3.2 Experimental Procedures

3.3.2 a) Treatment

On each occasion, an animal handler standing in the pen containing the lambs, caught the lamb to be treated and presented it to the operator who stood on the outside of the pen. Each lamb was held firmly with its rump resting on the pen wall, allowing easy access to the scrotal area for castration to be carried out (Fig. 3.2).

3.3.2 b) Groups

Control Handling (Controls)*(n=9)*: The operator gently manipulated the testes for approx 30 seconds mimicking the handling associated with castration. The lamb was then returned to the pen.

ACTH*(n=9)*: Lambs were injected intrajugularly with synthetic adrenocorticotropin (Synacthen; Ciba pharmaceutical's, Auckland) at a dose of 14 µg/kg body weight. This dose was the same as that used by Mellor and Murray (1989b) to elicit a large cortisol response in lambs.

Ring*(n=9)*: A constricting rubber ring (Allflex New Zealand Ltd., Palmerston North) was applied to the neck of the scrotum using an elastrator (Elastrator Ltd; Blenheim; Fig. 3.3) after ensuring that both testes were distal to the ring.

Short Scrotum *(n=9)*: Lambs had a rubber ring applied to the scrotum with both testes proximal to the ring ensuring that they were pushed up against the abdominal wall. This treatment has sometimes been mistakenly called cryptorchidism.

Burdizzo 10 Seconds (Burd 10) *(n=9)*: The spermatic cord and associated scrotal tissue on each side were crushed by the jaws of the burdizzo (The Ritchey Nipper, Ritchey Tagg Ltd. Masham, Nth Yorkshire, England. Fig. 3.2

and Fig. 3.3) for 10 seconds. Crushing across the full width of the scrotum, which would cause necrosis distal to the crush, was avoided.

Burdizzo 1 Second (Burd 1) *(n=9)*: The same method as for the Burd 10 group was used except that the burdizzo was applied for 1 second to each spermatic cord and associated tissues.

Burdizzo 10 seconds plus Ring (Burd 10 + Ring) *(n=9)*: The burdizzo was applied in the same way as for the Burd 10 group. However after application of the burdizzo, a rubber ring was applied as close as possible to the crush marks caused by the burdizzo. Due to the size of the lambs the burdizzo was used before the ring to reliably crush each spermatic cord. This was different from the method described by Molony *et al.* (1993) and Kent *et al.* (1995) who applied the ring first.

Burdizzo 5 Seconds plus Ring (Burd 5 + Ring) *(n=9)*: The same treatment as for the Burd 10 + Ring group was used except the burdizzo was only applied for 5 seconds.

Burdizzo 1 Second plus Ring (Burd 1 Ring) *(n=9)*: The same treatment as for the Burd 10 + Ring group was used except the burdizzo was only applied for 1 second.

Local Anaesthetic (LA) Scrotal Neck Control (LA SC Control) *(n=9)*: Local anaesthetic (Nopaine. 2% Lignocaine hydrochloride, Ethical Agents, Auckland) was injected into 3 sites around the scrotal neck and then massaged to disperse the local anaesthetic 15 minutes before treatment. One ml was injected into the anterio-medial surface of the scrotum and 0.5 ml into each lateral surface. The lambs were otherwise handled in the same way as Control lambs.

LA Spermatic Cord Control (LA CD Control) *(n=9)*: Local anaesthetic (0.5 ml) was injected into each spermatic cord 15 minutes before treatment. The lambs were otherwise handled in the same way as Control lambs.

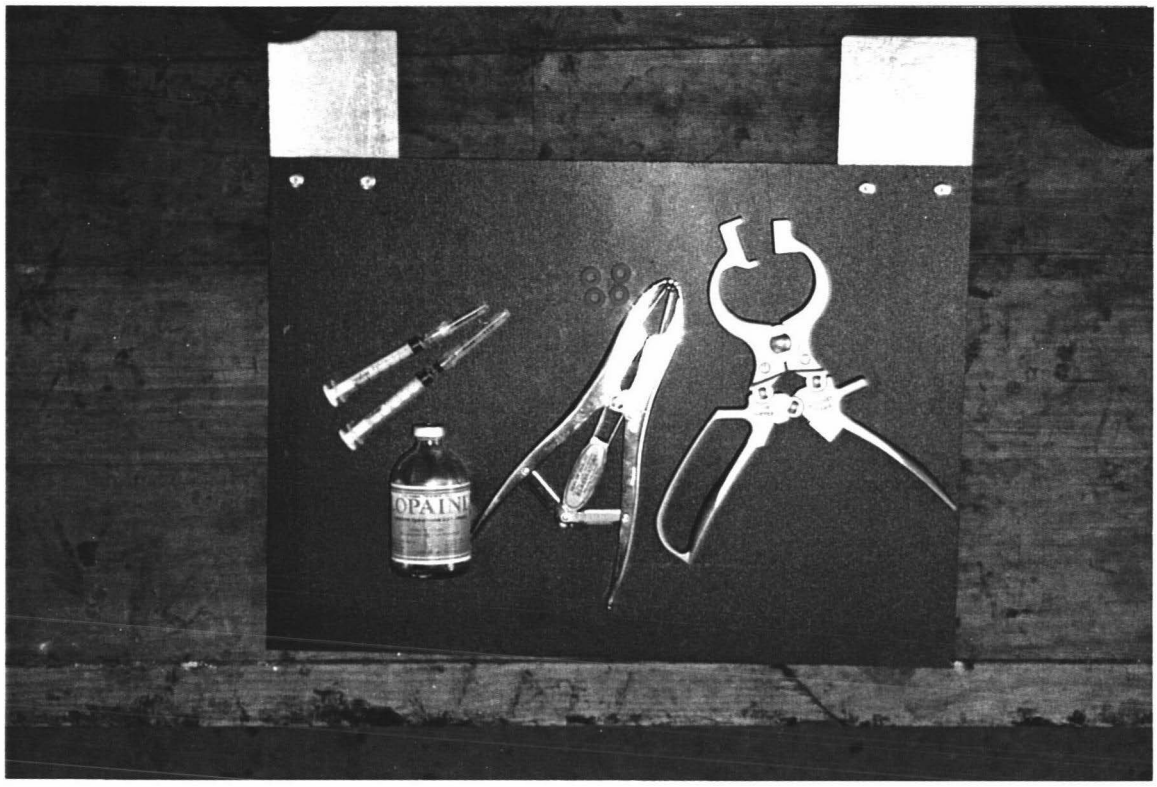


Fig. 3.3 Equipment used in this study. From left; 2 ml syringes, Lignocaine local anaesthetic, elastrator, rubber rings, burdizzo (The Ritchey Nipper).

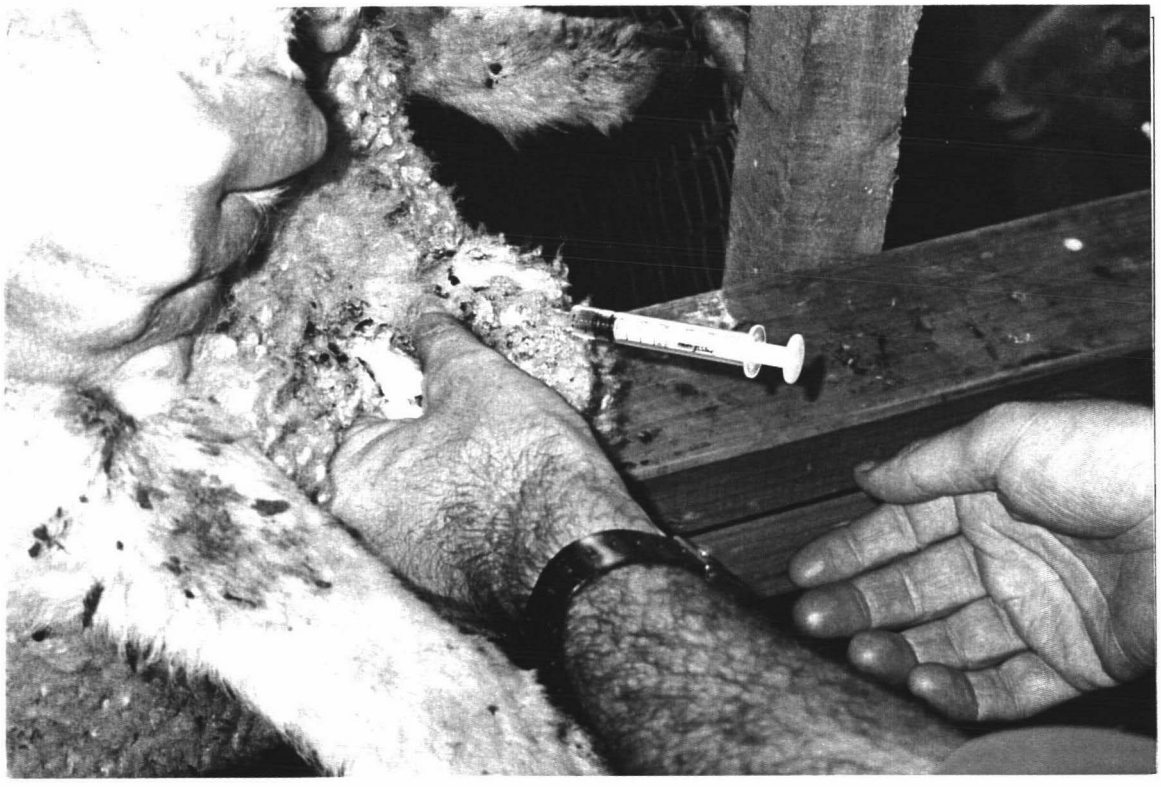


Fig. 3.4 Injection of local anaesthetic into a testis.

LA Scrotal Neck and Spermatic Cord Control (LA SC+CD Control) *(n=9)*: Local anaesthetic was injected into the scrotal neck in the same way as LA SC Control lambs, and then into each spermatic cord in the same way as LA CD Control lambs. The lambs were then handled in the same way as Control lambs.

LA Testes Control (LA T Control) *(n=9)*: The lambs had 0.5 ml local anaesthetic injected into each testis through the caudal pole, 15 minutes before treatment (Fig. 3.4). The lambs were then handled in the same way as Control lambs.

Ring LA SC *(n=9)*: Local anaesthetic was injected into the scrotal neck as in LA SC Control lambs, and 15 minutes later a rubber ring was applied in the same way as for Ring lambs.

Ring LA CD *(n=9)*: Local anaesthetic was injected into each spermatic cord in the same way as for LA CD Control lambs, and 15 minutes later a rubber ring was applied in the same way as for Ring lambs.

Ring LA SC+CD *(n=9)*: Local anaesthetic was injected into both spermatic cords and the scrotal neck 15 minutes before treatment in the same way as described for LA SC+CD Control lambs, and 15 minutes later a rubber ring was applied in the same way as for Ring lambs.

Ring LA T *(n=9)*: Local anaesthetic was injected into the testes in the same way as LA T Control lambs, and 15 minutes later a rubber ring was applied in the same way as for Ring lambs.

Burdizzo 10 Seconds LA SC (Burd 10 LA SC) *(n=8)*: Local Anaesthetic was injected into the scrotal neck in the same way as LA SC Control lambs, and 15 minutes later the burdizzo was applied for 10 seconds in the same way as for Burd 10 lambs.

Burdizzo 10 Seconds LA CD (Burd 10 LA CD) *(n=9)*: Local anaesthetic was injected into the spermatic cords in the same way as LA CD Control lambs, and

15 minutes later the burdizzo was applied in the same way as for Burd 10 lambs.

Burdizzo 10 Seconds + Ring LA SC (Burd 10 + Ring LA SC) *(n=9)*: Local anaesthetic was injected into the scrotal neck in the same way as for LA SC Control lambs, and 15 minutes later the lambs were treated in the same way as Burd 10 + Ring lambs.

Burdizzo 10 Seconds + Ring LA CD (Burd 10 + Ring LA CD) *(n=9)*: Lambs were injected with local anaesthetic in each spermatic cord in the same way as for LA CD Control lambs, and 15 minutes later the lambs were treated in the same way as Burd 10 + Ring lambs.

3.3.3 Blood Sampling

Blood samples (5 ml) were taken by jugular venipuncture 15 minutes before treatment (which was just before any local anaesthetic administration), immediately prior to treatment, and at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 minutes after treatment. On each occasion, blood sampling usually took less than 15 seconds to complete. It was noted whenever difficulty in obtaining a blood sample extended this period.

Blood samples, collected in heparinised vacutainers, were centrifuged within 30 minutes. The plasma was separated and stored at -20 ^{o}C until radio-immunoassay could be carried out.

3.3.4 Assessment of Efficacy of Castration

All lambs were examined three to four weeks after castration. Lambs were checked for the presence or absence of a scrotum, and if this was present its contents were scored from 1 to 5. A score of 1 was given if the testes had completely atrophied and 5 if the testes were apparently undamaged. The presence or absence of any infections was noted.

3.3.5 Radio-Immunoassay

The cortisol concentration in each plasma sample was determined using a non-extraction tritium radio-immunoassay method. The samples were assayed with standard plasma samples to determine within and between assay coefficients of variation which were 10% and 13% respectively. The lowest detectable concentration was 1.5 nmol/l.

Plasma samples were diluted with Hormone Free Plasma (HFP), combined with tritiated cortisol (Amersham), cortisol antiserum (Endocrine Sciences RIA Reagents Ltd, Calabasas CA), bovine gamma globulin (Serva, Heidelberg), and bovine serum albumin (Boehringer, Manheim). The samples were then incubated overnight at 4^{o}C to allow competitive binding to take place. The cortisol bound to the antibody was then separated from the unbound cortisol using polyethylene glycol 4000 (PEG) (BHD Ltd., Poole) precipitation. The precipitate (after being centrifuged) was resuspended in 1 ml of double distilled water and then combined with scintillation fluid. The radioactivity was then determined using a scintillation counter. Using a set of standard known concentrations of cortisol and knowing that the amount of radioactivity is inversely proportional to the concentration of cortisol in the sample, the concentrations of cortisol in the plasma could be determined (Lester 1991).

3.3.6 Presentation of Results

The cortisol concentrations for each group are presented as the mean concentrations ± the standard error of the mean (SEM) at each sample time. To correct for individual variation, the pre-treatment value was subtracted from the concentration in every subsequent sample. Graphical presentation is therefore expressed in terms of change in the mean plasma cortisol concentrations from the values at 15 minutes before treatment (t = -15 minutes).

Comparisons were made between concentrations in different groups at each time point using the Students t test assuming unequal variation (Microsoft® Excel V 5.0). This test allowed for unequal variations within the different groups at each specific time. The same comparisons were also made using the Students t-test assuming equal variance (Microsoft® Excel V 5.0) and in most cases there was no difference in the outcome.

Presented also are the integrated cortisol responses. The integrated cortisol response is defined as the area between a horizontal line drawn through the pre-treatment concentration (at -15 minutes) and the cortisol response curve during the period when the concentrations were greater than that pre-treatment value (Mellor and Murray, 1989b). Comparisons were made using the Student's t test assuming unequal variance. Any difference noted as being significant is significant with $p<0.05$.

3.4 RESULTS

Lambs found to have cortisol concentrations that were two standard deviations above the mean value for that group at a minimum of three consecutive sampling times, were excluded from the comparisons. A complete list of lambs excluded and their cortisol responses are provided in Appendix 1. Two of the lambs excluded may have exhibited very high cortisol responses because they had their necks clipped immediately prior to treatment. There was no apparent explanation for the elevated cortisol responses in any of the other lambs.

Mean plasma cortisol concentrations found here were generally about half those reported by Kent *et al.* (1995) and Molony *et al.* (1993), but were similar to those reported by Lester *et al.* (1991a,b) and Mellor and Murray (1989a). These differences could have been due to assay techniques, breed of lambs and also the previous experiences of the lambs. However the relative cortisol

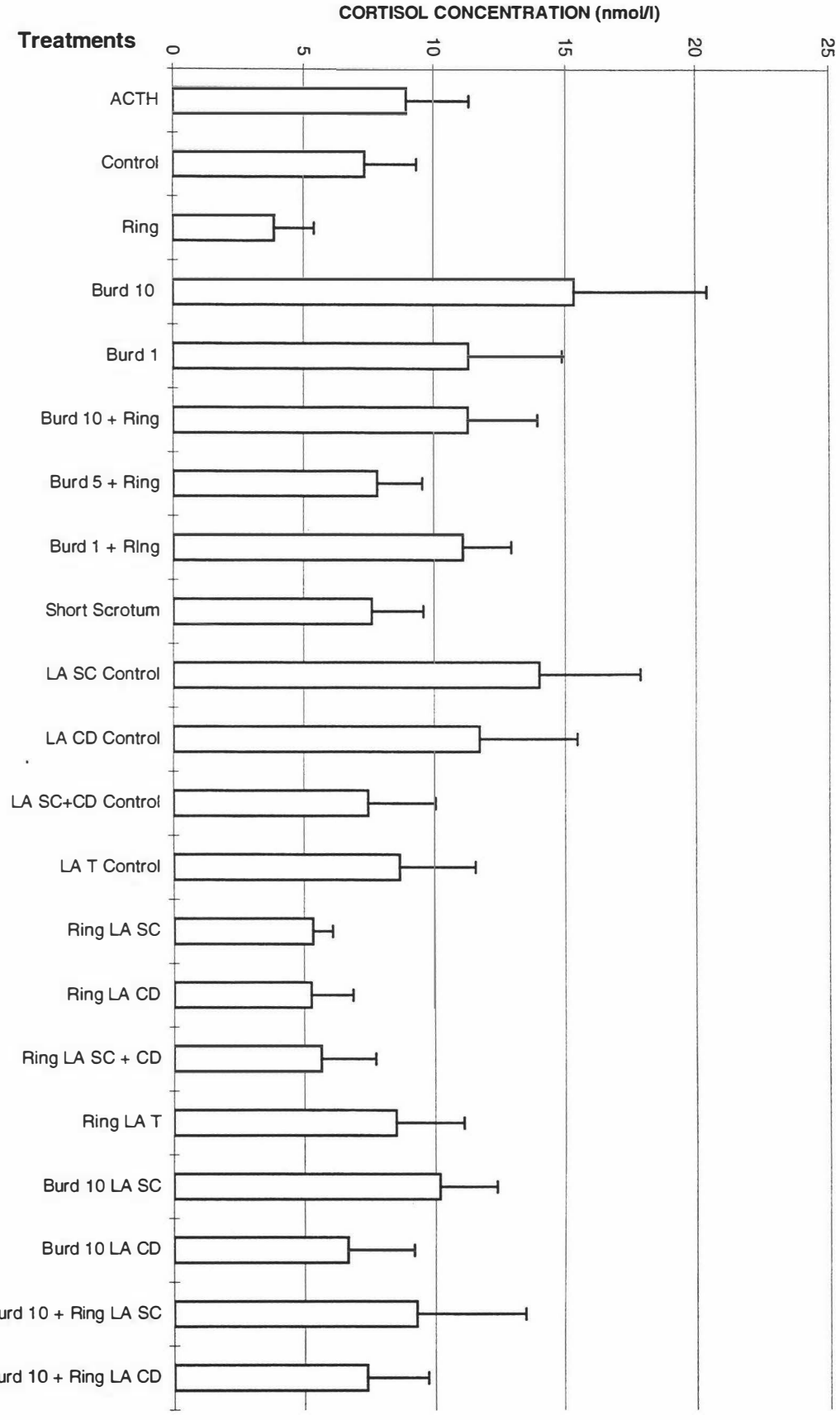


Fig 3.5: Pre- treatment cortisol concentrations (mean + SEM) at t = -15 minutes. There were no significant differences between pretreatment cortisol concentrations using Two way analysis of variance (ANOVA) .

responses to the different castration techniques may be assessed by reference to the cortisol responses to ACTH, control handling, and/or other standard castration/tailing techniques such as the ring, enabling comparison of the results obtained by different groups of workers.

Mean (± SEM) pre-treatment cortisol concentrations are presented in Fig. 3.5.

3.4.1 Cortisol Responses

Control: Control lambs showed a transient increase in mean plasma cortisol concentrations after initial handling. The mean concentrations returned to values that were not significantly different from pre-treatment values by 45 minutes after handling (Fig. 3.6).

ACTH: Mean plasma cortisol concentrations in lambs injected with ACTH approached maximum values after 30 minutes and remained there until 90 minutes after injection. Although the cortisol concentrations declined after 90 minutes, they had not returned to pre-treatment values by 4 hours after treatment (Fig. 3.6). The maintenance of plateau values between 30 and 90 minutes after treatment suggests that the ACTH injection elicited maximal cortisol secretion until 90 minutes after injection.

Ring: Lambs castrated with a ring displayed a mean cortisol response with highest values at 60 and 90 minutes after treatment. Concentrations remained elevated above pre-treatment values for 150 minutes after application of the ring. The mean cortisol concentrations were significantly higher than Control values between 30 and 120 minutes after treatment (Fig. 3.7).

Short Scrotum: Short Scrotum lambs exhibited a mean cortisol response that followed a similar pattern to ring castrated lambs, with highest values occurring

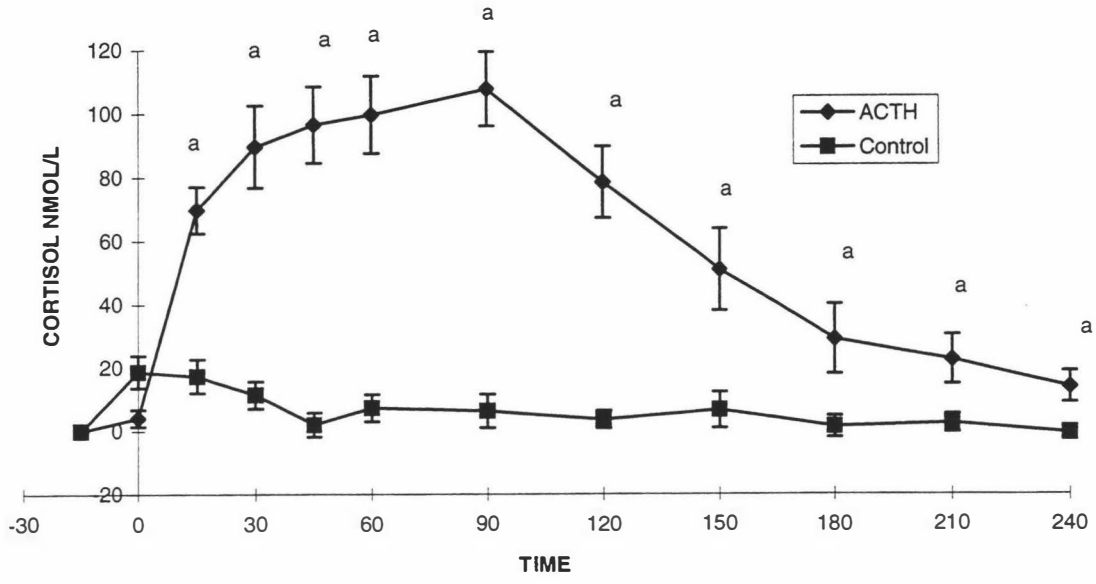


Fig 3.6: Change in plasma cortisol concentrations of lambs in response to control handling or ACTH injection. Super script above mean value indicates significant difference ($p<0.05$) from Control values (a).

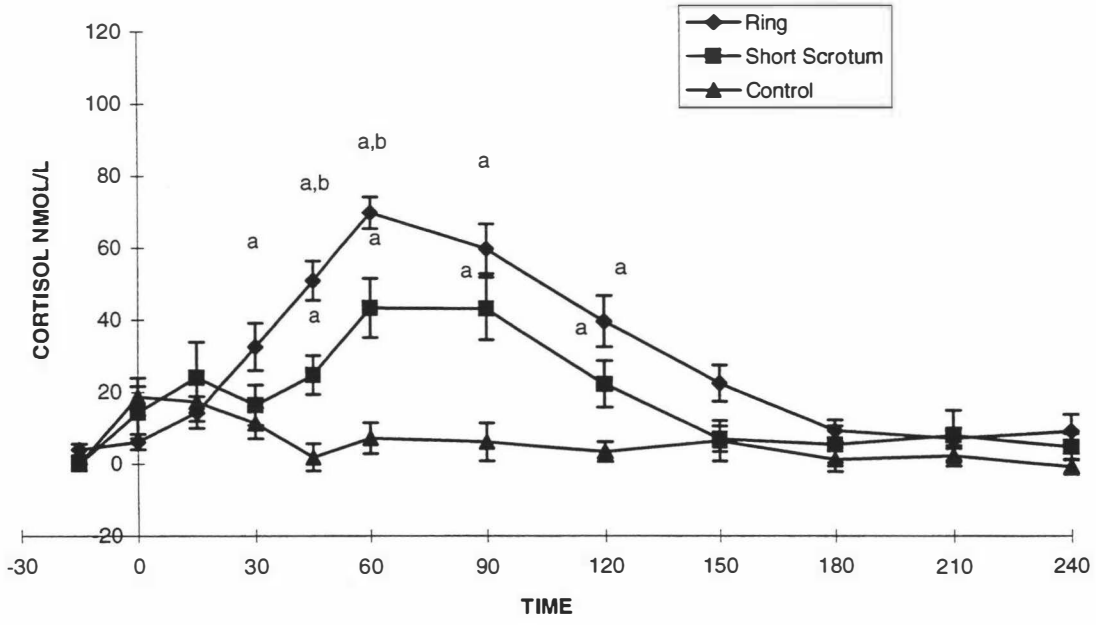


Fig 3.7: Change in plasma cortisol concentrations in response to control handling, short scrotum creation, or rlng castration. Superscript above mean indicates significant difference from; (a) Control, and (b) Short Scrotum ($p<0.05$).

between 60 and 90 minutes after treatment. Mean cortisol concentrations remained significantly above pre-treatment values for 120 minutes after treatment (Fig. 3.7). Mean cortisol concentrations of Short Scrotum lambs were significantly less than those of Ring lambs between 45 and 60 minutes after treatment, and were significantly higher than those of Control lambs between 45 and 120 minutes after castration.

Burdizzo 10 Seconds: Burd 10 lambs exhibited a mean cortisol response that approached maximal concentrations 30 minutes after treatment. That was 30 minutes before maximal concentrations were reached in Ring lambs. Concentrations remained elevated until 210 minutes after treatment, declining to pre-treatment values at 240 minutes. The concentrations in Burd 10 lambs were significantly higher than those in Ring lambs at 15 and 180 minutes after treatment (Fig. 3.8). The cortisol responses showed a large amount of variation between individual lambs in the Burd 10 group (Fig. 3.8). The cortisol concentrations in this group were significantly higher than corresponding Control values between 15 and 210 minutes after treatment.

Burdizzo 1 Second: Burd 1 lambs had a mean cortisol response that reached maximal concentrations at 30 minutes and returned to pre-treatment values by three hours after treatment. The mean concentrations were significantly less than the corresponding values in Ring lambs at 60 and 90 minutes after treatment (Fig. 3.9). They were also significantly higher than concentrations in Control lambs between 15 and 150 minutes after treatment. The cortisol responses of Burd 1 and Burd 10 lambs were only significantly different at 180 minutes after treatment (Fig. 3.10).

Burdizzo 10 Seconds plus Ring: Burd 10 + Ring lambs exhibited a mean cortisol response that reached maximal concentrations 30 minutes after castration which was half an hour before the peak in Ring lambs. Maximal cortisol concentrations of Burd 10 + Ring lambs were not significantly different from Ring lambs, however mean concentrations of Burd 10 + Ring lambs were significantly higher at 15 and 30 minutes after treatment. From one hour after

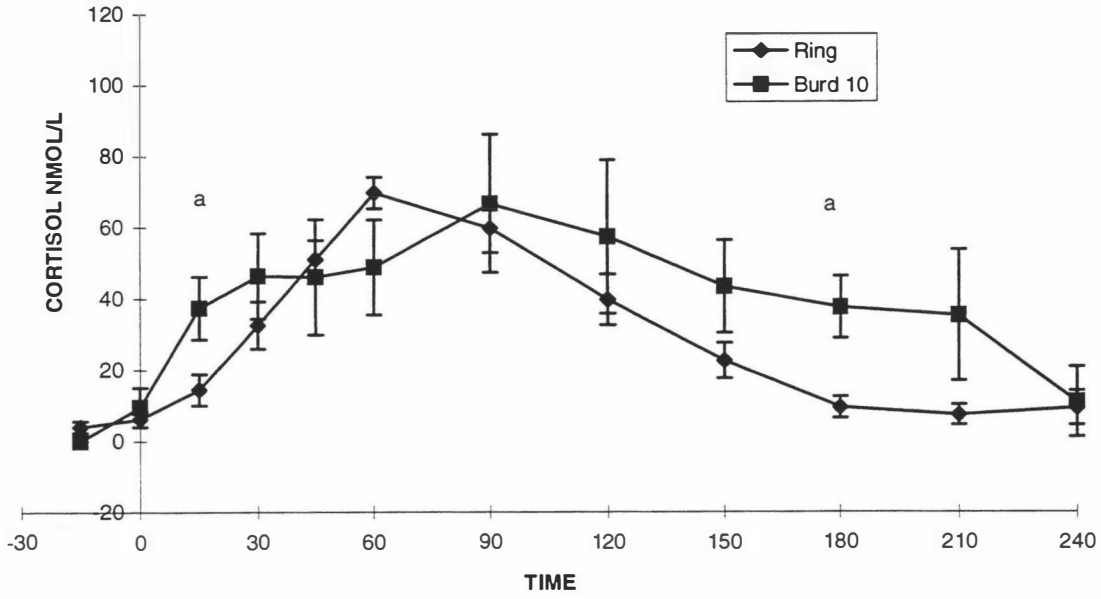


Fig 3.8: Change in plasma cortisol concentrations in lambs in response to castration by a burdizzo (10 seconds) or a ring. Superscript (a) above mean value indicates a significant difference ($p<0.05$) from Ring values.

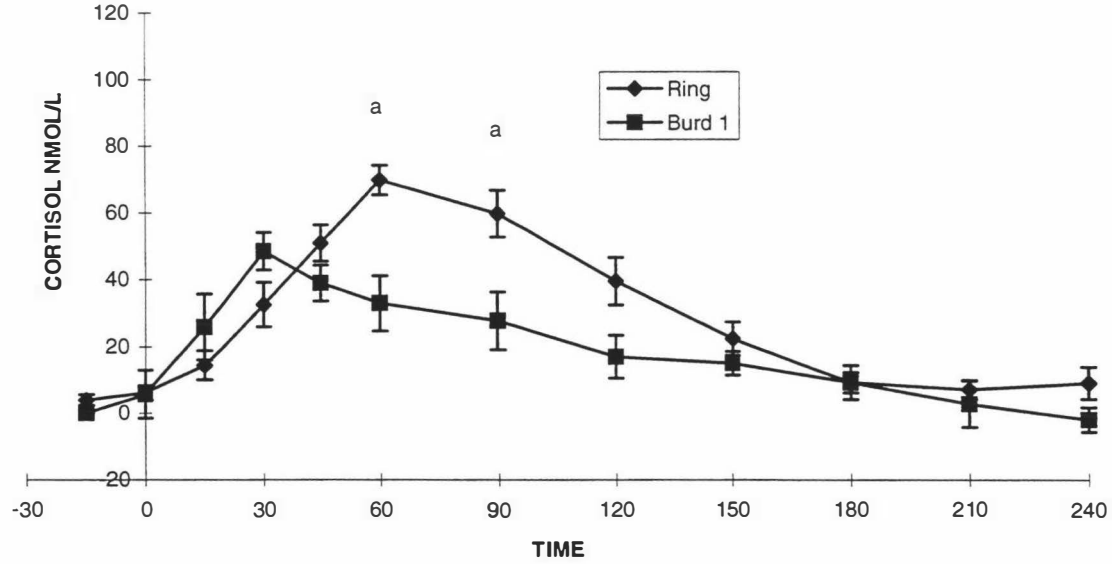


Fig 3.9: Change in plasma cortisol concentration of lambs in response to castration with a burdizzo (1 second), or a ring. Superscript above mean value indicates significant difference ($p<0.05$) from Burd 1 (a).

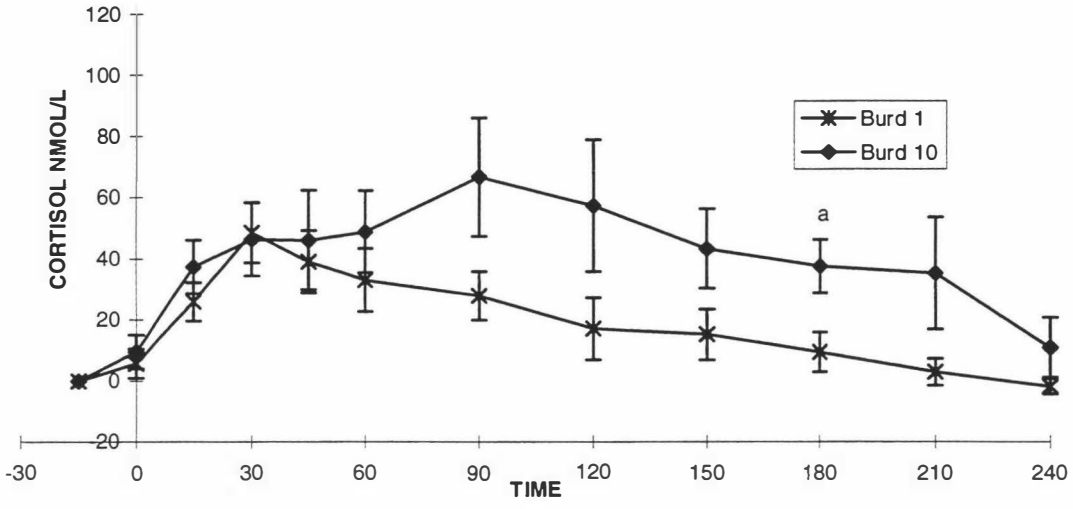


Fig. 3.10: Change in plasma cortisol concentrations in lambs in response to castration with a burdizzo (1 or 10 seconds). Superscript(a) indicates significant difference (P<0.05) from Burd 1 lambs.

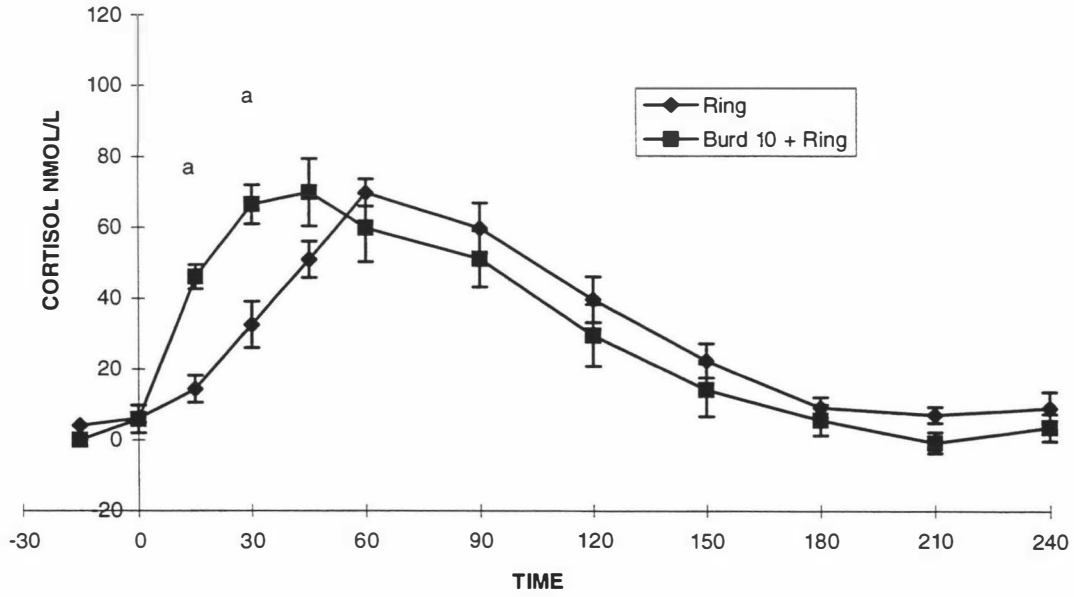


Fig 3.11: Change in plasma cortisol concentrations in lambs in response to castration with a Burdizzo 10 seconds+Ring, or a ring. A superscript (a) above a mean value indicates a significant difference (p<0.05) from Ring.

treatment, the concentrations were similar in Ring and Burd 10 + Ring lambs and they both then decreased to pre-treatment values by 150 minutes after treatment (Fig. 3.11). The cortisol concentrations in Burd 10 + Ring lambs were significantly higher than the corresponding values of Controls between 15 and 150 minutes after treatment.

Burdizzo 5 Seconds plus Ring: Burd 5 + Ring lambs exhibited a mean cortisol response that reached maximal concentrations at 15 minutes after treatment. Although mean cortisol concentrations tended to be lower in Burd 5 + Ring than in Burd 10 + Ring lambs, they were not significantly so. However, cortisol concentrations of Burd 5 + Ring lambs were significantly higher than in Ring lambs 15 minutes after treatment (Fig. 3.12). They were also significantly higher than Control values between 15 and 150 minutes after treatment.

Burdizzo 1 Second plus Ring: The mean cortisol response of Burd 1 + Ring lambs was very similar to that shown by Burd 10 + Ring lambs, and at no time were the mean concentrations significantly different. Mean plasma cortisol concentrations of Burd 1 + Ring lambs were significantly higher than in Ring lambs at 15 and 30 minutes after treatment (Fig. 3.13), and significantly higher than in Control lambs between 15 and 150 minutes after treatment.

Local Anaesthetic Controls (SC, CD, SC+CD, T): The mean cortisol concentrations in all Local Anaesthetic Control lambs were significantly higher than pre-treatment values at 0 minutes and at 15 minutes after treatment, except in LA T Control lambs. However, in all LA Control Groups, cortisol concentrations remained at or less than pre-treatment values from 30 minutes after treatment. At 30 minutes after treatment LA SC Control lambs had mean plasma cortisol concentrations that were significantly less than in Control lambs, and also at 30 minutes after treatment, LA T Control lambs had significantly lower values than all of the other LA Control and Control lambs (Fig. 3.14).

Ring LA SC, Ring LA SC+CD, Ring LA T: All of these groups showed a slight but significant elevation in mean plasma cortisol concentrations at 45-60 minutes after treatment. All Ring LA groups (except Ring LA CD) had

significantly lower values than Ring lambs between 30 and 150 minutes (Fig. 3.15). Ring LA SC lambs had significantly higher plasma cortisol concentrations than LA SC Control lambs between 30 and 60 minutes after treatment and significantly higher concentrations than Control lambs at 45 minutes after treatment. Ring LA SC+CD, and Ring LA T mean cortisol responses were not significantly different from their respective LA controls or Control lambs at any time after treatment.

Ring LA CD: Ring LA CD lambs had a mean cortisol response that reached maximal values at 45 to 60 minutes. These values, although slightly lower than those shown by Ring lambs were not significantly lower. There were large variations in the cortisol responses in lambs in the Ring LA CD group (Fig. 3.16). These lambs had a mean cortisol response that was significantly higher than LA CD Control and Control lambs between 45 and 90 minutes after treatment.

Burdizzo 10 Seconds LA SC, Burdizzo 10 Seconds LA CD: The mean cortisol responses of lambs in these two groups reached maximal concentrations at 30 minutes and remained elevated until 120 minutes after treatment. Levels returned to pre-treatment values at 240 minutes after treatment. Although concentrations tended to be lower between 60 and 240 minutes after treatment in Burd 10 LA SC and Burd 10 LA CD lambs than in Burd 10 lambs, there were no significant differences between them (Fig. 3.17).

Burdizzo 10 Seconds plus Ring LA SC, Burdizzo 10 Seconds plus Ring LA CD: Burd 10 + Ring LA SC or Burd 10 + Ring LA CD lambs exhibited a mean plasma cortisol response that reached maximal concentrations at 15 minutes after treatment. Burd 10 + Ring LA SC and Burd 10 + Ring LA CD lambs had significantly lower cortisol concentrations than Burd 10 + Ring lambs from 30 minutes to 120 minutes after treatment (Fig. 3.18). Maximal values of these two groups were significantly less than the corresponding maximal values in Burd 10 lambs.

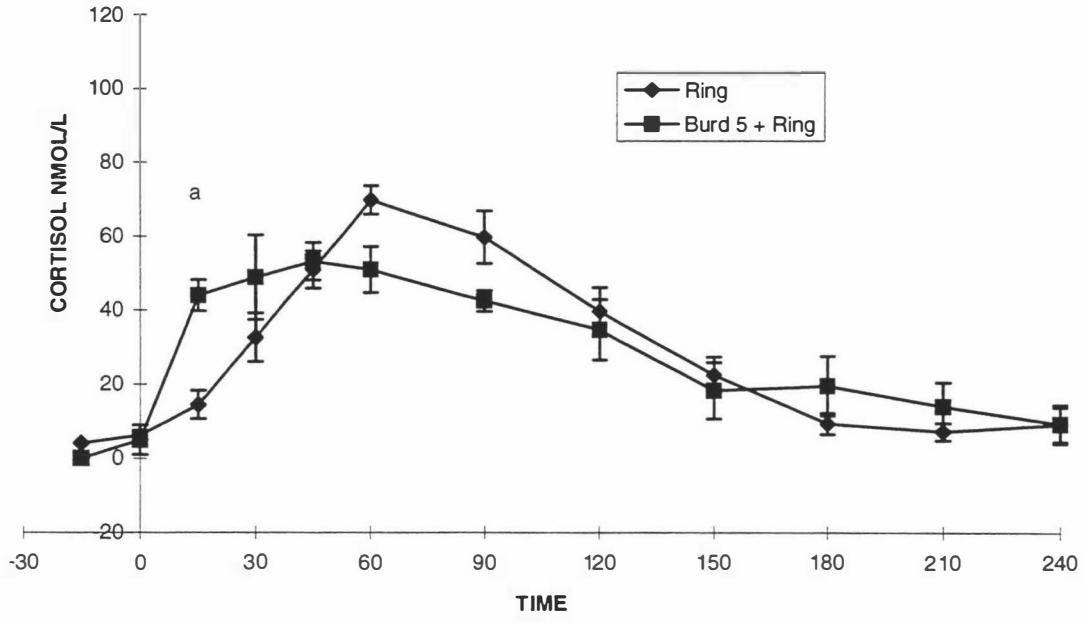


Fig 3.12: Plasma cortisol concentrations in lambs in response to castration with Burdizzo 5 seconds + Ring or a ring. A superscript (a) above a mean value indicates a significant difference ($p<0.05$) from Ring.

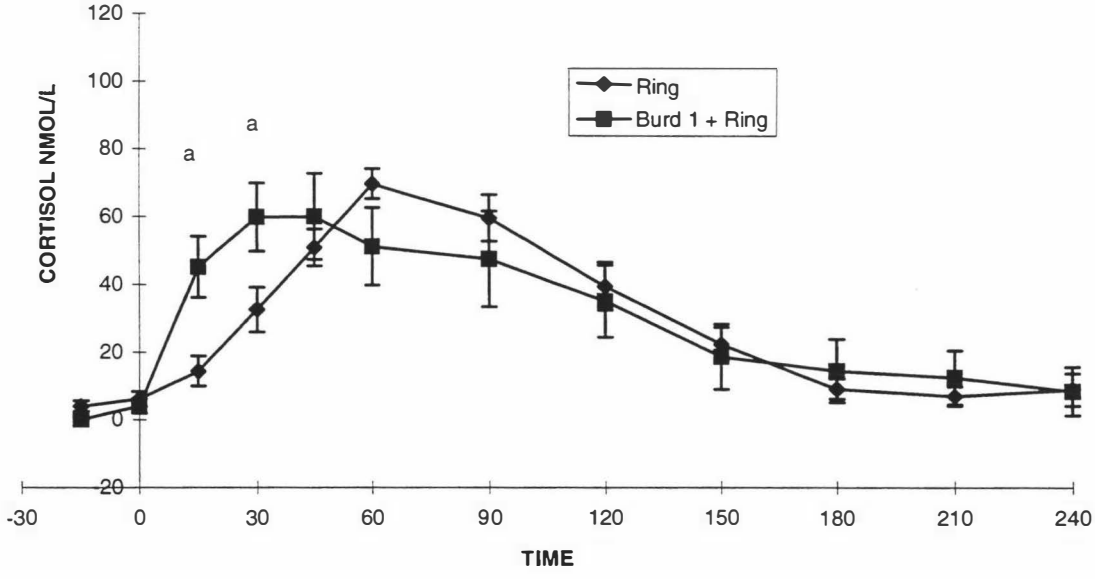


Fig 3.13: Change in plasma cortisol concentrations in lambs in response to castration with a burdizzo 1 second + Ring, or a ring alone. Superscript (a) indicates a significant difference ($p<0.05$) from Ring.

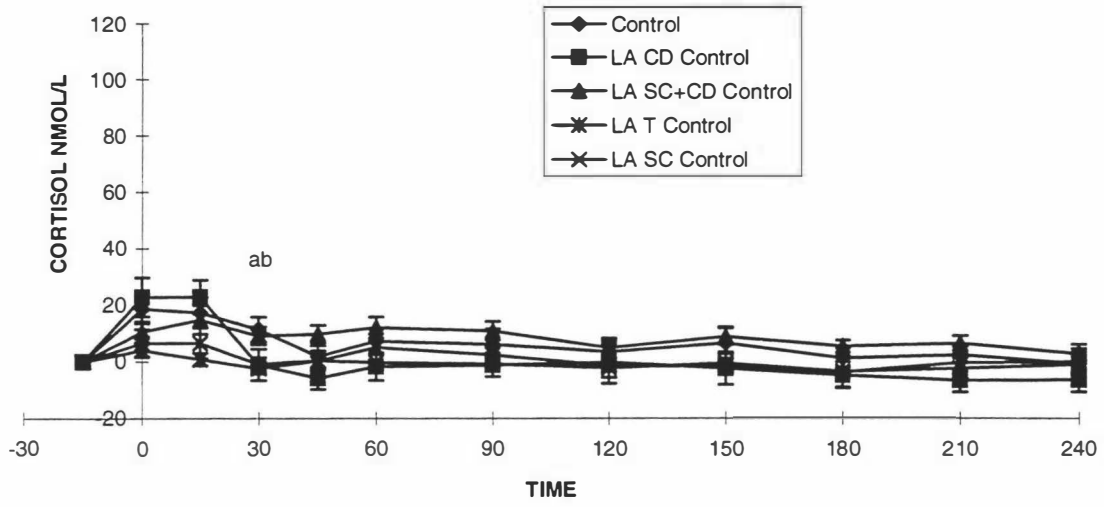


Fig 3.14: Change in plasma cortisol concentrations of lambs injected with local anaesthetic(LA) in the scotal neck(SC), spermatic cord(CD), testes(T), or spermatic cord and scrotal neck(SC+CD).

Super scripts above mean values indicate significant differences between; (a) LA SC Control and Control lambs and, (b) LA T Control and all other Control and LA Control Lambs.

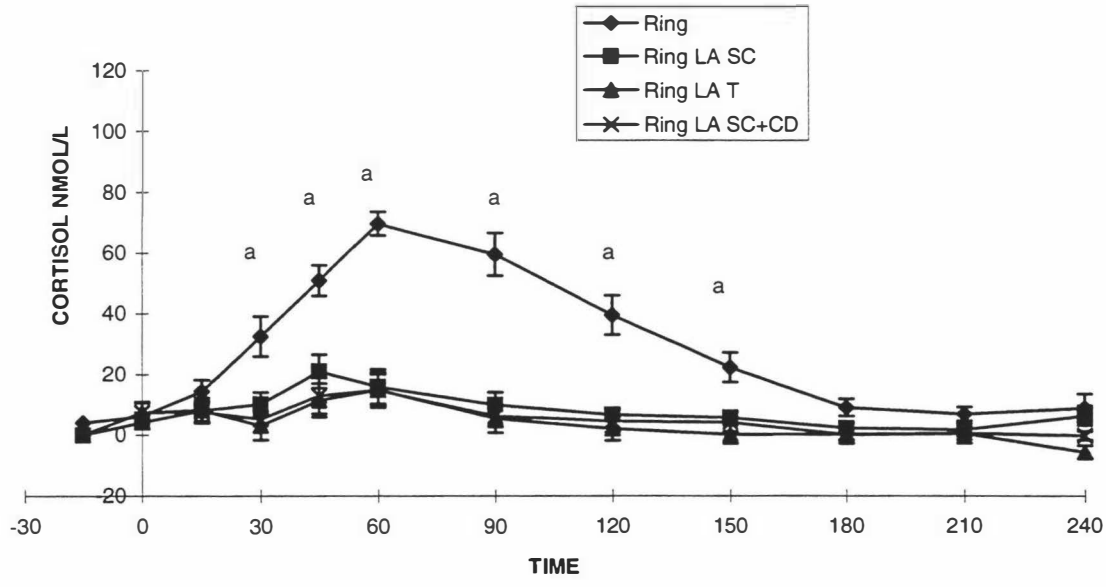


Fig 3.15: Change in plasma cortisol concentrations of lambs in response to ring castration with or without prior local anaesthetic (LA) injected into the scrotum (SC), scrotum plus spermatic cords(SD + CD), or testes (T).
A super script (a) above a mean value indicates a significant difference ($p<0.05$) between Ring lambs and Ring LA SC, Ring LA SC+CD, or Ring LA T lambs.

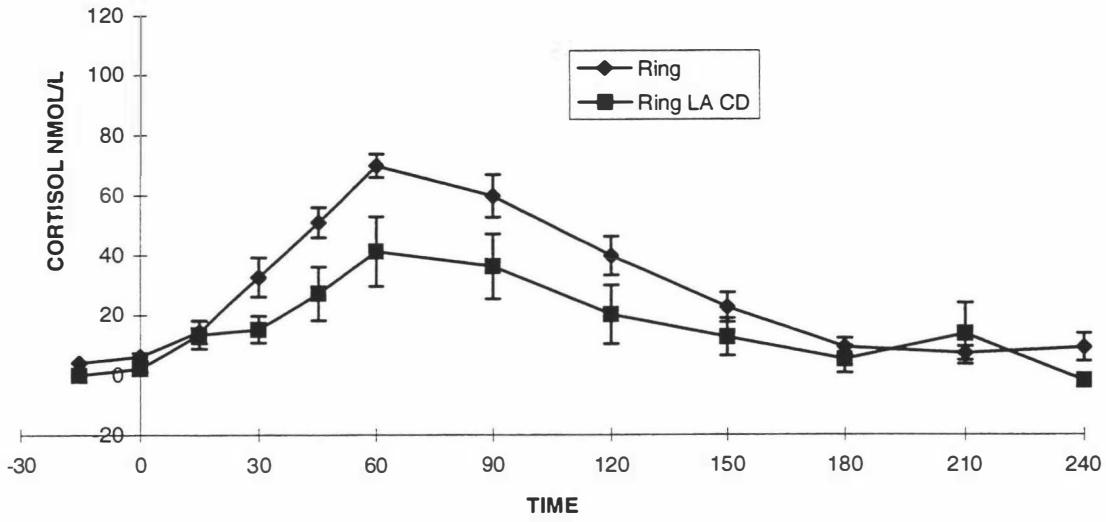


Fig 3.16: Change in plasma cortisol concentration in lambs in response to ring castration with or without prior local anaesthetic in the spermatic cord (CD). There were no significant differences due to large variation within Ring LA CD group.

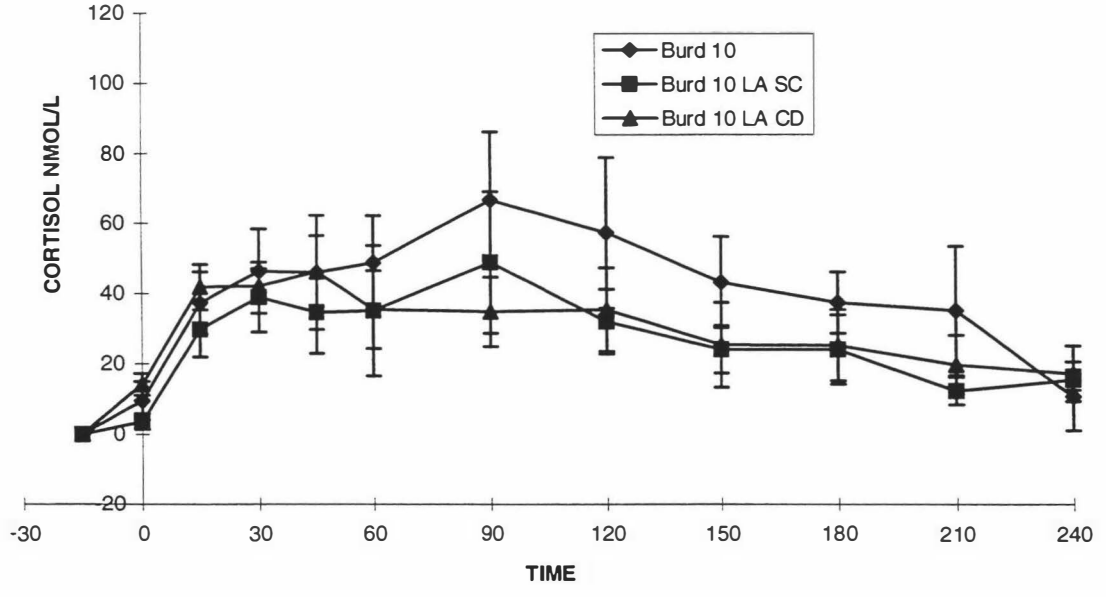


Fig 3.17: Change in plasma cortisol concentrations in lambs in response to burdizzo (10 seconds) castration with or without prior local anaesthetic injected into the scrotum or spermatic cords. There were no significant differences.

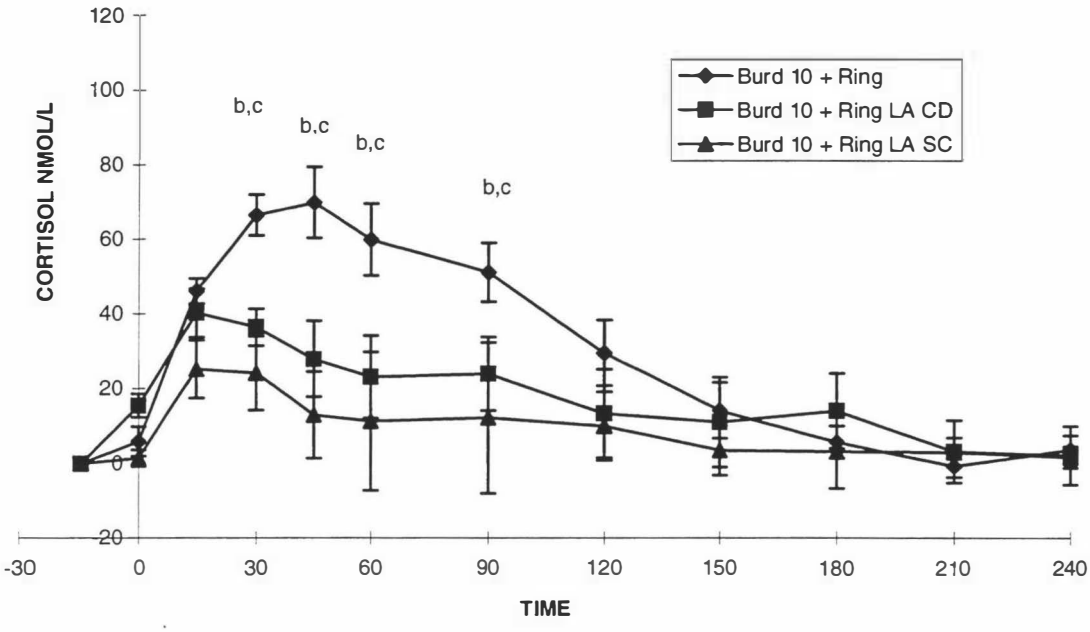


Fig 3.18: Change in plasma cortisol concentrations in response to Burdizzo 10 seconds + ring castration with or without prior local anaesthetic injection into the scrotum (SC) or spermatic cords (CD).
Superscripts above mean value indicates significant difference ($p<0.05$) from (b) Burd 10 + Ring LA SC, or (c) Burd 10 + Ring LA CD lambs.

3.4.2 Integrated Cortisol Responses.

The mean integrated cortisol responses (± SEM) for all the groups are shown in table 3.1.

Control and ACTH: The mean integrated cortisol response was significantly lower ($p<0.01$) in Control than in ACTH lambs (Fig. 3.19). The mean integrated cortisol response of ACTH lambs was significantly higher than in all other groups except for Burd 10 lambs (Fig. 3.19).

Ring, Short Scrotum The mean integrated cortisol response for Ring lambs was significantly greater than for Control lambs, but not significantly different from Short Scrotum lambs (Fig. 3.19). Short Scrotum lambs exhibited a mean integrated cortisol response that was significantly higher than Control lambs (Fig 3.19).

Ring, Burd 10 + Ring, Burd 5 + Ring, Burd 1 + Ring: The mean integrated cortisol responses of the three Burd + Ring groups were not significantly different from each other, or from the mean responses of Ring lambs (Fig. 3.19).

Ring, Burd 10, Burd 1: Although Burd 1 lambs had a mean integrated cortisol response that was approximately half of that exhibited by Burd 10 lambs, there was not a significant difference between them. There was a large amount of variation between individual integrated cortisol responses of Burd 10 lambs which affected the comparison. There was no significant difference in the mean integrated cortisol responses between Burd 10, Burd 1 and Ring lambs (Fig. 3.19).

Local Anaesthetic controls: The mean integrated cortisol responses of all LA control lambs were not significantly different from the integrated response of Control lambs. However, the mean integrated cortisol response of LA T Control lambs was significantly lower than LA SC + CD Control lambs (Fig. 3.20), otherwise there were no significant differences between LA Control lambs.

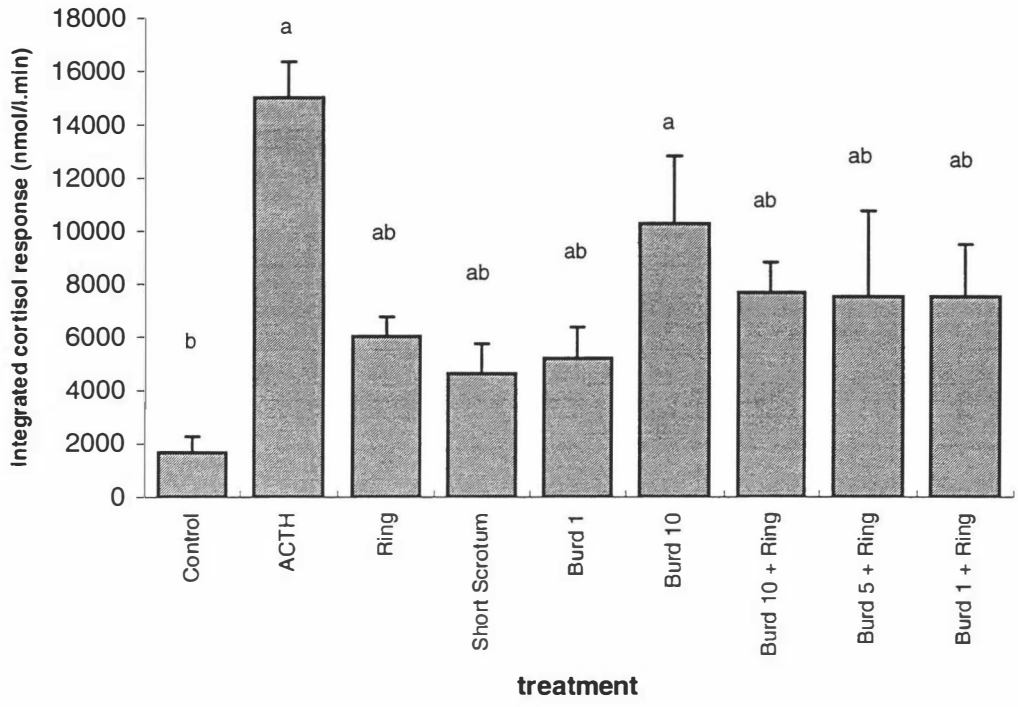


Fig 3.19 Integrated cortisol responses (mean ± SEM). Superscript indicates significant differences to (a) Control and (b) ACTH.

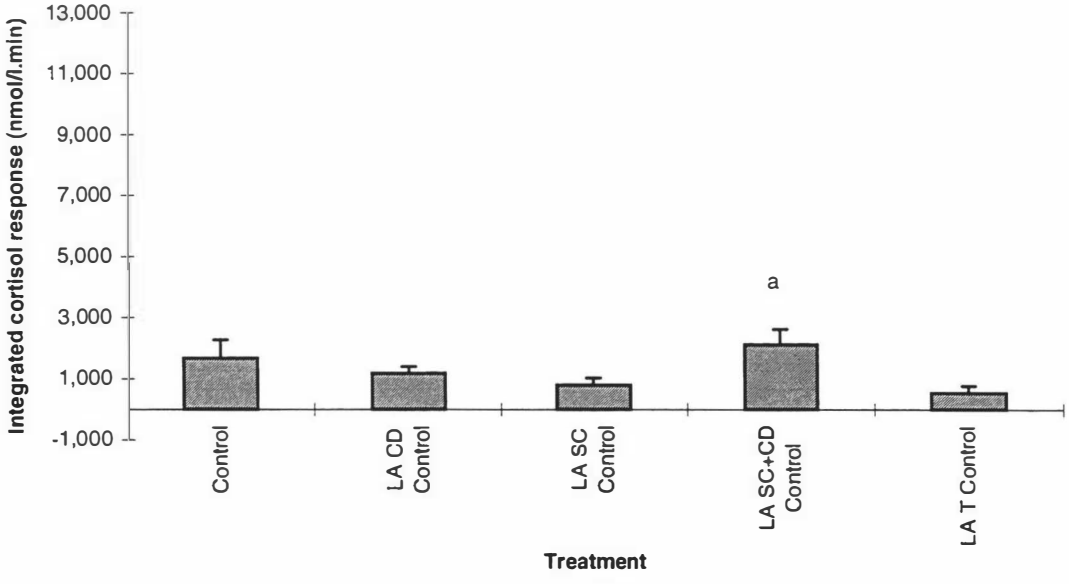


Fig 3.20: Integrated cortisol responses of Control and LA Control lambs. Superscript (a) indicates a significant difference ($p<0.05$) to LA T Control values.

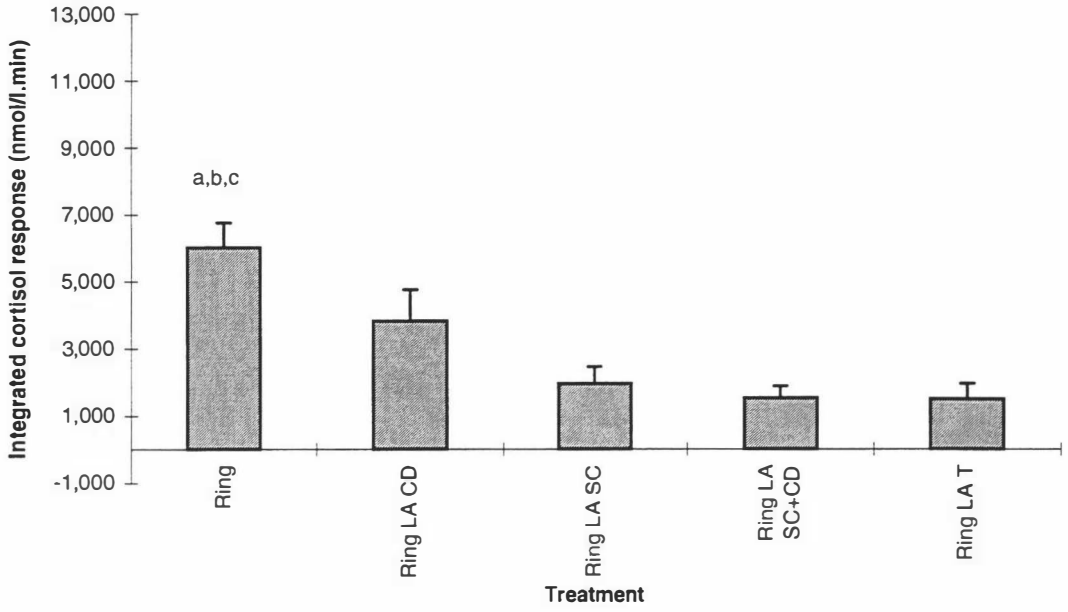


Fig 3.21: Integrated cortisol responses of lambs castrated with a ring, with or without pre-treatment with local anaesthetic. Superscripts indicate significant differences ($p< 0.05$) from; (a) Ring LASC, (b) Ring LA SC+CD, (c) Ring LA T values.

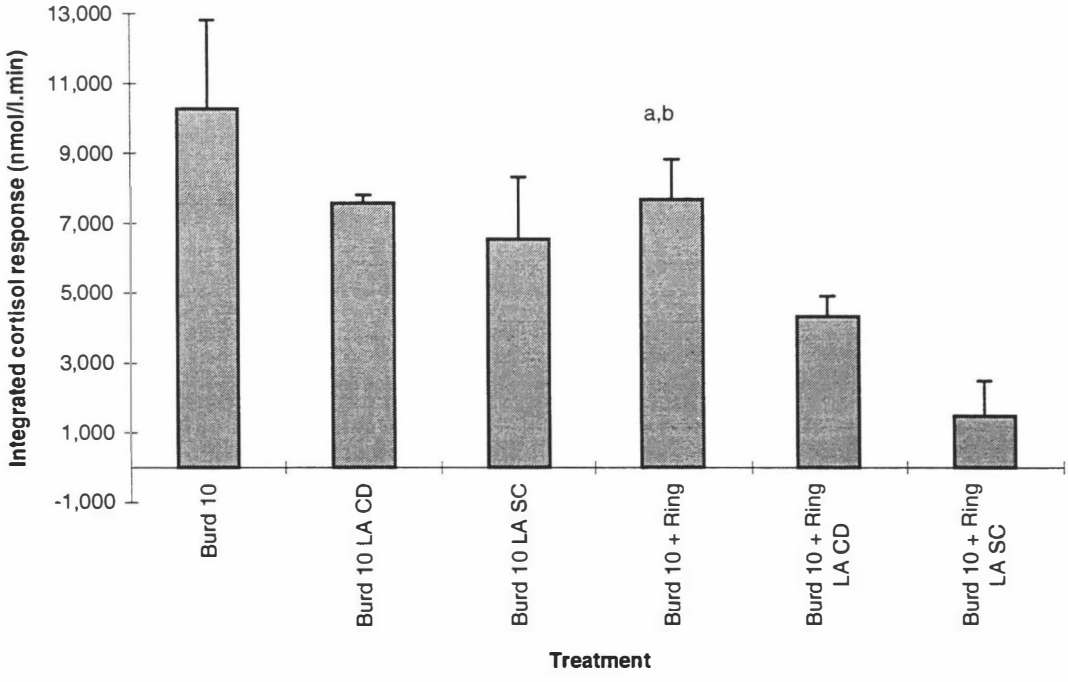


Fig 3.22: Integrated cortisol responses of lambs castrated by different methods.Superscripts indicate significant differences ($p<0.05$) from (a) Burd10+Ring LA CD, (b) Burd10+Ring LASC values.

Ring, Ring LA SC, Ring LA T, Ring LA SC+CD: The mean integrated cortisol responses for Ring LA SC, Ring LA T, and Ring LA SC+CD lambs were all significantly lower than the response of Ring lambs but not significantly different from each other (Fig. 3.21). They were also not significantly different from their respective LA controls or the response of Control lambs.

Ring, Ring LA CD: The mean integrated cortisol response of Ring LA CD lambs, although slightly lower, was not significantly different from that of Ring lambs (Fig. 3.21). The mean integrated cortisol response of Ring LA CD lambs was significantly higher than the response of LA CD Control lambs.

Burd 10, Burd 10 LA SC, Burd 10 LA CD: The mean integrated cortisol responses for Burd 10 LA SC and Burd 10 LA CD lambs were not significantly different from Burd 10 lambs (Fig. 3.22). The integrated cortisol responses of the Burd 10 LA SC and Burd 10 LA CD lambs were both higher than in their respective LA controls or in the Control lambs.

Burd 10 + Ring, Burd 10 + Ring LA SC, Burd 10 + Ring LA CD: The mean integrated cortisol responses for Burd 10 + Ring LA SC and Burd 10 + Ring LA CD lambs were both significantly lower than that of Burd 10 + Ring lambs (Fig. 3.22). The mean integrated cortisol responses were both higher than the values in their respective LA control or in the Control lambs.

TREATMENT	MEAN AREA (nmol/l.min)	SEM
ACTH	15,006	1,335
Control	1,668	598
LA CD Control	1,180	212
LA SC+CD Control	2,126	496
LA SC Control	798	230
LA T Control	540	234
Ring	6,022	735
Short Scrotum	4,623	1,111
Ring LA CD	3,822	932
Ring LA SC	1,959	505
Ring LA SC+CD	1,528	353
Ring LA T	1,498	467
Burdizzo 1 + Ring	7,518	1,962
Burdizzo 5 + Ring	7,518	3,215
Burdizzo 10 + Ring	7,672	1,140
Burdizzo + Ring LA SC	1,494	1,003
Burdizzo + Ring LA CD	4,327	576
Burdizzo 1	5,191	1,171
Burdizzo 10	10,348	2,536
Burdizzo LA SC	6,536	1,770
Burdizzo LA CD	7,568	233

Table 3.1 The integrated cortisol responses for lambs given different castration or control treatments. The mean area under the cortisol curve ± SEM. LA = local anaesthetic, SC = scrotum, CD = spermatic cord, T = testes, SC+CD = scrotum + spermatic cord.

3.4.3 Efficacy of Castration Methods

The scrotum/testes of all lambs castrated with a ring, with or without burdizzo application, had sloughed off when examined 3 weeks later, so that castration was deemed to have been successful. The distal part of the scrotum was absent in Short Scrotum lambs three weeks after application.

Burdizzo application did not always successfully cause atrophy of both testes. Two lambs in the Burd 10 group, and one in the Burd 1 group had one testis that, after palpation, was considered to be intact.

	Ring(n=33)	Burdizzo 10 Sec(n=20)	Burdizzo 1 Sec(n=7)	Burdizzo and ring(n=37)
total	100%	90%	86%	100%
partial	0	10%	14%	0

Table 3.2 Percentage of lambs that were successfully castrated (scrotum removed or atrophy score 1 or 2 for both testes) or partially castrated (scrotum still present and atrophy score between 3 and 5 for one or more testes) at a minimum of three weeks after treatment. Testis atrophy score between 1 and 5, 1 = absent, 5= undamaged.

3.5 DISCUSSION

The Burdizzo did not reduce the magnitude or duration of the plasma cortisol response to ring castration.

The burdizzo (1, 5 or 10 seconds), did not influence significantly the duration or magnitude of the cortisol response to Ring castration. This is contrary to the findings of Kent *et al.* (1993,1995) who showed that the burdizzo, placed after application of a rubber ring, reduced the magnitude and duration of the cortisol response normally exhibited by ring castrated and tailed lambs. This apparent discrepancy between studies is probably due to a difference in the method of application of the burdizzo, however other differences were also present.

In the present study, the burdizzo was applied twice, once for each spermatic cord (which is the conventional method of burdizzo application), before a ring was applied. Presuming that the burdizzo crush does crush neural tissue, this method of burdizzo application left an area of undamaged, innervated and vascularised scrotal tissue between the two crushes which, when the burdizzo is used as the sole method of castration, prevents sloughing of the scrotum. The area of uncrushed tissue probably allowed noxious sensory input from hypoxic scrotal tissue, located distal to the ring and crushed areas, via still active afferent nerves contained in the uncrushed tissue. In a more recent report (Kent *et al.* 1995), it was made clear that the burdizzo was applied once across the whole width of the scrotum, distal to, and after ring application. This method of burdizzo application is likely to have significantly reduced the chance that afferent nerves within the scrotum remained intact and thereby prevented access of noxious sensory input from scrotal and testicular tissue to the CNS. Indeed, lambs castrated and tailed with this method exhibited a plasma cortisol response that was significantly lower and of a shorter duration than that exhibited by lambs castrated and tailed with rings alone (Kent *et al.* 1995).

In the present study, castration only was investigated. Tailing and the use of the burdizzo on the tail may have contributed to the difference between the results presented in this chapter and those presented by Kent *et al.* (1993;

1995). However, it is noteworthy that other workers have found that lambs castrated and tailed with rings exhibited a cortisol response that was not significantly different from lambs castrated only with a ring (Lester *et al.* 1991a). To further investigate the use of the burdizzo plus a ring to both castrate and tail dock, a further study was undertaken which is described in Chapter 4.

In contrast to the studies by Kent *et al.* (1993;1995), in the present study the burdizzo was applied before the application of the rubber ring. This difference is unlikely to influence the amount of nociception and the corresponding cortisol responses because it has been shown that neural activity, in the spermatic cord at least, can continue to pass through tissue compressed by a rubber ring for 90 minutes after application (Cottrell and Molony, in press). Hence, nociception resulting from the burdizzo crush is likely to be able to pass through the ring so that the presence of the ring before or after the burdizzo is applied is likely to be inconsequential.

In this study, the burdizzo was applied twice, hence the actual duration of insult was 20 seconds (in Burd 10 + Ring lambs). This is in contrast to Kent *et al.* (1995) who applied the burdizzo once for 10 seconds.

Another contributing factor to the differences between the present study and those described by Kent *et al.* (1993,1995) is the burdizzo instruments used were different. Differences in design and wear can result in different pressures being applied, different jaw dimensions, and differences in the gap between each jaw when fully closed. These differences may result in incomplete nerve crushing, and differing amounts of tissue damaged. The size of lambs in relation to the burdizzo may also be a factor.

Although it is clearly not possible to determine the exact cause of the different results obtained here and by Kent *et al.* (1993,1995), application of the burdizzo to each side of the scrotum (present study) compared to its application across the whole scrotum (Kent *et al.* 1993, 1995), as discussed earlier, seems the most probable explanation.

The nature of the cortisol response to Burdizzo + Ring Castration

The nature of the cortisol response to burdizzo plus ring castration without the confusing influences of tailing was observed in this study. The cortisol response to burdizzo plus ring castration was different from that to ring castration in the initial phase when the cortisol concentrations in burdizzo plus ring lambs were significantly higher than in ring lambs during the first 30 minutes. The higher initial values in the Burd + Ring lambs probably reflected an initial large barrage of noxious sensory input caused by the crushing of spermatic cords and associated scrotal tissue by the burdizzo and the further insult of adding a tight rubber ring to the damaged tissue. The cortisol concentrations presumably took longer to increase in response to ring castration because the noxious sensory input is likely to be caused by a more protracted development of hypoxia, hypercapnia, hypoglycemia, hyperkalaemia, acidosis and a build up of inflammatory mediators released from hypoxic and damaged cells (Wood *et al.* 1991; MacIver and Tanelian 1992; Cottrell and Molony, in press). Assuming that the burdizzo successfully prevented further transmission of nociception through nerves that it crushed, it is somewhat surprising that the cortisol response exhibited was as great as that caused by ring castration. Even though some tissue remained intact between the two crushed areas, it might be thought that at least some nociception from ischaemic tissue distal to each crush would have been prevented. This observation questions whether indeed the burdizzo was successful in damaging the nerves sufficiently to prevent continued nociception, or whether the barrage from the damaged nerve continued for longer than expected. Further laboratory experiments involving measurement of the activity of isolated nerves after crushing are required to clarify this.

It is likely that the later part of the cortisol response to Burd + Ring castration was influenced by the amount of noxious sensory input caused by the ring because the plasma cortisol concentrations exhibited by Ring and Ring + Burd 10 lambs were very similar from 60 minutes after treatment. This probably indicates that the increasing hypoxia/anoxia caused death of nociceptors and

hence a reduction followed by abolition of nociception from tissues distal to the ring.

The duration of burdizzo application (1,5, or 10 seconds) did not have any significant effects on the cortisol response to burdizzo + ring castration. Although no significant differences were observed, the cortisol responses tended to be lower after application of the burdizzo for 1 second than after application for 5 or 10 seconds.

The scrotum and testes are both sources of noxious sensory input during ring or burdizzo + ring castration

The cortisol response to ring castration is likely to be due to noxious sensory input from the testes and scrotum.

It is presumed that one of the major contributing factors to the cortisol response is noxious sensory input, probably nociception, from ischaemic tissues distal to and directly underneath the ring. Tissue ischaemia is likely to result in hypoxia, hypercapnia, hyperkalaemia, acidosis and release of inflammatory mediators. These conditions may be expected to stimulate nociceptors (Cottrell and Molony, in press). It has been shown that there are polymodal receptors in dog testes that are responsive to chemical changes in the scrotum (Kumazawa and Mizumura 1980a, Kumazawa et al. 1987). That there are noxious sensory inputs which cause the cortisol responses to ring or burdizzo plus ring castration, is supported by the fact that the cortisol responses to these procedures can either be reduced (Ring LA CD, Burd + Ring LA CD lambs) or abolished (Ring LA SC, Ring LA T, Ring LA SC+CD, Burd + Ring LA SC lambs) with local anaesthetic depending on the injection site or sites.

The results of the present study suggest that scrotal tissue was a major source of noxious sensory input after ring castration but that the relative amounts of sensory input from both scrotal and testicular tissues were difficult to quantify. This was in contrast to the suggestion of Wood *et al.* (1991) that the main source of nociceptor activity after ring castration was testicular, rather than

scrotal tissue. Before castrating and tailing lambs with rubber rings, they injected local anaesthetic into all three sites used in the present study (scrotal neck, spermatic cords, testes), and also into the tail. This produced a period of an apparent absence of pain-induced distress that was longer than the expected duration of anaesthesia. Surprisingly it was suggested that occlusion of blood supply by the ring would impede the normal clearance of the local anaesthetic from the intratesticular and not the scrotal or spermatic cord injections. Thus the testes were considered to be the major sites of noxious input after ring castration of lambs (Wood *et al.* 1991). This idea may be challenged on the following grounds.

- Any local anaesthetic located distal to the ring would have been expected to be retained whatever the site of injection.

- The duration of anaesthesia expected by the group (one hour), lasted almost as long as the cortisol response exhibited by unanaesthetised lambs in the same study.

- Results from the dye study discussed in Chapter 2 suggested that intratesticular injections of local anaesthetic could also have anaesthetised parts of the scrotum.

These arguments, together with the following observations made in the present work suggest that there was significant scrotal and testicular involvement in the noxious inputs which followed ring and indeed, burdizzo plus ring castration.

- The Short Scrotum technique, which elicited a significant cortisol response that was less than that caused by Ring castration, probably causes noxious sensory input (due to ischaemia and concurrent anoxia) from scrotal tissue only and not testicular tissue. It has recently been shown that the short scrotum technique does not stimulate the nociceptors of the testis or pampiniform plexus (Cottrell and Molony, in press).

- Lambs with local anaesthetic injected into the spermatic cord before Ring castration and Short Scrotum lambs exhibited similar plasma cortisol

responses. As sensory input via the spermatic cord, at least distal to, and in the area of the injection site should have been abolished in the Ring LA CD lambs, it may be assumed that testicular nociceptors did not contribute to the cortisol response in these lambs. Therefore, the plasma cortisol response exhibited by these lambs would have been mainly due to the activity of scrotal nociceptors. It must be noted that this injection technique was difficult and produced some uncertainty as to where the local anaesthetic was actually injected. The results described in Chapter 2 indicated that most injections were likely to get into the spermatic cord.

- Local anaesthetic injected into the scrotal neck virtually abolished the cortisol response to ring and burdizzo plus ring castration. Although this could suggest that the scrotum is the sole site of nociceptor activity after ring application, this type of injection put sufficient anaesthetic into the scrotal area to effect both the scrotum and its contents. Such injections may be capable of anaesthetising the tunica albuginea of the testis and the spermatic cord as discussed in Chapter 2.

- Although intratesticular injections abolished the cortisol response to ring castration suggesting that the testes were the only source of noxious sensory input, the results of the study discussed in Chapter 2 showed that leakage of local anaesthetic from each testis could have resulted in the scrotum being anaesthetised as well. It is important to note that in the study discussed in Chapter 2 the extent of leakage after ring application was not examined.

- The testes have been shown to be a source of sensory input after ring application in lambs by Cottrell and Molony (in press). Mechanoreceptor fields were present within the deep tissue of the testis and pampiniform plexus, and were most active immediately after application of the rubber ring with activity decaying exponentially for 90 minutes when it ceased. Mechanoreceptors with a high threshold were classified as nociceptors, as the compression required to excite these receptors is known to be painful in humans (Cottrell and Molony, in press). Polymodal receptors, which are considered to be nociceptors, with axons in the superior spermatic nerve are

located in the tunica vaginalis visceralis in the dog (Kumazawa and Mizumura 1980a). Testicular polymodal receptors in the dog have been shown to be sensitive to chemical stimulation (Kumazawa and Mizumura, 1980b). It is likely that polymodal receptors which are sensitive to chemical stimulation are also present in the lamb.

Although both scrotal and testicular tissue are likely to be sources of noxious input, the relative amounts of nociception from the two sources caused by ring castration may still be difficult to determine from the results of this study. The difficulty arises because it has been shown that when there is more than one source of noxious input, the resulting cortisol response is not equal to the sum of the cortisol responses seen when each source of noxious input is isolated (Lester *et al.* 1991a). It is therefore, only possible to conclude that both the scrotum and testes are sources of noxious input after ring castration.

The use of the burdizzo alone elicited a cortisol response that was no less in magnitude or duration than that caused by the ring.

The use of the burdizzo alone for 1 or 10 seconds elicited a cortisol response that was not significantly less in magnitude or duration than that elicited by ring castration. This result was similar to that reported by Kent *et al.* (1995). There was large variation between cortisol responses in the Burd 10 group. Some lambs showed a cortisol response that was slightly higher than controls, others showed a large prolonged response. This variation in burdizzo castrated lambs was also reported by Kent *et al.* (1995). In the present study it is likely that this reflects a varying amount of scrotal skin left intact between the two areas of crushed scrotal tissue. If there was minimal or no tissue left intact, or that which was intact did not contain any major afferent nerve trunks, it is likely that the sensory input that passed to the CNS and correspondingly the cortisol response elicited, would have been minimal. However, if there was a substantial amount of scrotal tissue left undamaged which was also highly innervated, then the active nociceptors in the hypoxic tissue, caused by the

crush, may still have contained viable afferent innervation enabling the lambs to experience pain. The variation between lambs may also have been due to a variation in the size of lambs, the technique of the operator, the pressure exerted by the burdizzo and the size of the burdizzo.

The long duration of responses seen in lambs that show large cortisol responses, may have been due to a number of things. First, the active nociceptors may have remained viable for a longer time because the blood supply was not completely disrupted to the area. Secondly, it is possible that inflammatory mediators such as interleukins and histamines released from the sites of cell damage could have caused secondary sensitisation of the innervated area, increasing the activity and number of active nociceptors. This is supported by the fact that prior treatment with NSAIDs, that inhibit the production of sensitising and inflammatory mediators, has been shown to reduce both the magnitude and the duration of the cortisol response to burdizzo castration (Molony 1993). It is also possible that the prolonged cortisol response was not due to noxious sensory input, but mediators being released into the systemic system stimulating the hypothalamic-pituitary-adrenal axis (HPA) independently from nociceptors (Kent *et al.* 1995). This is discussed further in the next section.

The results of this study provide little evidence to support sensory-independent stimulation of the HPA after any treatment except for burdizzo alone.

The results of this and other studies provide little evidence to support the theory that sensory-independent stimulation of the HPA (as discussed in Chapter 1) influences the cortisol response after castration and other tissue removals. The only exception in this study is the cortisol response to burdizzo castration. This will be discussed later. The following are reasons why the theory of sensory-

independent stimulation of the HPA is unlikely to be applicable when castrating lambs using the ring or burdizzo plus ring.

- The cortisol response to ring castration was abolished by local anaesthetic administration to the testes, scrotum, or scrotum + spermatic cords. A smaller but marked reduction in the cortisol response to burdizzo plus ring castration was obtained by injecting local anaesthetic into the scrotum or spermatic cords. If a large proportion of the cortisol response was influenced by sensory independent stimulation of the HPA after ring or burdizzo plus ring castration, then abolition of the cortisol response using local anaesthetic to inhibit sensory input would not have occurred in LA Ring lambs. The small residual cortisol response in LA burdizzo + ring lambs could be due to some direct humoral effects on the HPA, but this seems unlikely (see below).

- It is unlikely that inflammatory mediators, which have been postulated to be the factors that stimulate the HPA independently of sensory input, released from damaged cells distal to the ring would gain access to the HPA. This is because the ring prevents the flow of blood to and from any tissues distal to it. However, there may be some release of these inflammatory mediators from tissue just proximal to the ring which would also be expected to have a disrupted blood supply.

Because local anaesthetic injected into the spermatic cord or into the scrotum did not reduce the cortisol response to burdizzo castration it may be thought that this indicates that stimulation of the HPA occurred independently of noxious sensory input. However there are reasons why this may not be the case.

- Anaesthesia of the area may have been incomplete. The spermatic cord injection has been shown to be ineffective in abolishing the cortisol response to ring castration due to the probable large amount of noxious input from the unanaesthetised scrotum.

- The period of local anaesthesia after burdizzo castration will be less than that obtained after ring castration because of the absence of a ring which

prevents the normal clearance of local anaesthetic. This will not be a factor in the initial part of the cortisol response when local anaesthetic would be expected to be effective.

- In a preliminary report by Molony (1993) it has been shown that the cortisol response to burdizzo castration can be reduced using local anaesthetic infiltration of the area. This indicates that at least some, if not all, of the cortisol response results from noxious sensory input.

- It has also been shown that prior application of NSAIDs can reduce the cortisol response to burdizzo castration to the same extent as prior local anaesthetic injection (Molony 1993). Although direct analgesic action on spinal cord is possible, the reduction of the cortisol response may indicate that the hyperalgesia caused by the inflammatory mediators acting on nociceptors was inhibited by NSAIDs, or alternatively that stimulation of the HPA by inflammatory mediators independently of sensory input was prevented by NSAID action inhibiting such mediator production. Prior injection of both local anaesthetic and NSAIDs markedly reduced the cortisol response to burdizzo castration. This is likely to indicate a reduction of noxious input caused by the initial damage of tissue, and inhibition of subsequent hyperalgesia caused by inflammatory mediators. Alternatively, if as proposed by Molony *et al.* (1995), it is the inflammatory mediators that stimulate the HPA independently of nociception, then noxious sensory input was likely to have been reduced as well as the stimulation of the HPA independently of noxious sensory input. It is however, not possible at present to determine which explanation is the actual mode of action, but, the first seems to be the most likely. That is because other evidence demonstrates that cortisol responses to other tissue removals such as dehorning in calves can be abolished by administration of local anaesthetics which act for about one third, one half, or all of the period during which cortisol concentrations are elevated after dehorning without local anaesthetic (Petrie *et al.* 1996b, C.P McMeekan, D.J. Mellor, K.J. Stafford, R.A Bruce, and R.N Ward, unpublished data).

It is however, surprising, that the scrotal neck injection had no effect on the cortisol response to burdizzo castration. If using a burdizzo to crush the spermatic cord does successfully prevent transmission of noxious input through nerves in that tissue, then it is surprising that the cortisol response to burdizzo castration was not successfully reduced by injecting local anaesthetic into the scrotum as complete blockage/disruption of transmission of nociception from tissues distal to the crush would be expected. However tissue of the scrotum and the spermatic cords cranial to the burdizzo cuts and anaesthetised areas may become sensitised by inflammatory mediators and become hyperalgesic. Although this may explain why local anaesthetic did not abolish the cortisol response it is still surprising that no reduction was seen. Therefore, sensory-independent stimulation of the HPA after burdizzo castration, although unlikely, cannot be ruled out using the results of this study.

The magnitude and duration of the cortisol response to ring, burdizzo or ring + burdizzo castration is not likely to be limited by the adrenal cortex.

Because the rate of increase in plasma cortisol concentrations was greater after injection of a large dose of ACTH than any other treatment in this study, it is likely that the maximum secretory rate of the adrenal cortex was not reached during the first 15-30 minutes after ring, burdizzo, or burdizzo plus ring castration.

That the rate of secretion, and the magnitude and duration of the cortisol responses presented in this study (except for exogenous ACTH injection) were not limited by tissues of the HPA is supported by the following observations.

1. It has been shown that in lambs, ring castration + tail docking can elicit a rate of cortisol secretion as great as that elicited by a large dose of ACTH in the first 30 minutes after treatment (Mellor and Murray 1989a). It has also been demonstrated that dehorning calves causes cortisol to be secreted at the same rate as after a large injection of exogenous ACTH (Petrie *et al.* 1996b). This demonstrates that endogenous CRF and ACTH secretory rates can cause a maximal cortisol secretory rate.

2. It has also been shown that in 3 week old lambs, plasma cortisol concentrations parallel plasma ACTH concentrations after injections of increasing amounts of CRF (Prandier *et al.* 1985). This also indicates that the secretory rate of ACTH from the anterior pituitary is unlikely to limit the cortisol secretory rate after a noxious experience.

The duration of the cortisol response is not likely to have been limited by the secretory capacity of the adrenal cortex. It has been demonstrated that in adult sheep, maximum cortisol concentrations can be maintained by infusion of exogenous ACTH for a period of at least 24 hours (D.J. Mellor, unpublished data). Other treatments in lambs such as surgical castration demonstrate that a larger cortisol response than any observed here can have a duration of at least eight hours (Lester *et al.* 1991b).

Efficacy and Welfare Advantages of the Different Methods of Castration.

Ring castration is one of the most effective methods of castration, with little chance of failure. It does not often result in infection, although sometimes it can predispose to tetanus. Lambs probably suffer a significant amount of distress (as indicated by the cortisol response) after ring castration. This can be abolished by using a prior application of local anaesthetic into the testes or the scrotum. As shown here, local anaesthetic injected into the testes or the scrotum probably anaesthetises at least partially both the testes and the scrotum.

In the study described by this Chapter, local anaesthetic was injected 15 minutes prior to treatment. However, it has been shown that 0.2 ml of intratesticular local anaesthetic only takes 2 minutes to block nociceptor activity in the testis (Cottrell and Molony, in press). It is unknown how long it takes for local anaesthetic to be effective in the scrotum. Due to the time taken for the noxious sensory input from ring application to be significant enough to elevate plasma cortisol concentrations (approx 15 minutes), it may be possible to give

an intratesticular injection of local anaesthetic at the same time as ring application, and still achieve a significant reduction in distress. However, after ring application distribution of the local anaesthetic may be inhibited by lack of blood flow and will then only be possible by natural diffusion helped by manual manipulation. It may be necessary to apply more local anaesthetic in a modified way to aid distribution in and around the testes. This method has many practical advantages; a) it is very easy, even for an unexperienced person, to inject local anaesthetic into the testicles and b) any infection resulting from unsterile needles would be less likely to spread from the testes or scrotum because of the constricting ring. A scrotal injection method is also possible although more care may be necessary to make sure enough local anaesthetic is applied around the scrotum to ensure complete anaesthesia of both the scrotum and testes. The use of local anaesthetic in these ways needs further study.

The use of the burdizzo alone in the conventional manner for castrating lambs is not regularly done in New Zealand. It can be less effective and more time consuming than using a ring with more room for operator error. This study has shown that the burdizzo used alone in the described manner can produce widely varying distress responses. Although the present discussion suggests that using the burdizzo alone across the whole width of the scrotum might be desirable to reduce distress, a major contraindication arises. When the scrotum is crushed across its width, necrosis, sepsis and granulation of scrotal tissue can occur. Therefore, the use of the burdizzo in this way as a practicable method of castration cannot be recommended on welfare grounds.

The use of the burdizzo on each spermatic cord before ring application, has been shown to be ineffective in reducing the magnitude or duration of distress (as indicated by plasma cortisol concentrations) associated with ring castration. However, use of the burdizzo in one application across the whole scrotum after ring application may be effective in reducing both the magnitude and duration of the cortisol response (Kent *et al.* 1995). The duration of application of the burdizzo produced small, but insignificant differences in cortisol responses in

the present study. However, when the burdizzo was applied across the width of the scrotum as in the study described by Kent *et al.* (1995), different application times may alter the cortisol response seen. Kent *et al.* (1995) have shown that application of the burdizzo across the width of the scrotum for 10 seconds after ring application decreased by more than one hour the time taken for the plasma cortisol concentrations to return to pre-treatment values. This was not the case in their earlier study (Kent *et al.* 1993) when the burdizzo was applied for 6 seconds after ring application and the cortisol response took 3 hours to return to pre-treatment values. In a practical farming situation the use of the burdizzo for 10 seconds on each lamb after ring application may be time consuming and frustrating when dealing with a large number of lambs.

CHAPTER 4: EFFECTS ON PLASMA CORTISOL CONCENTRATIONS OF CASTRATING AND TAILING LAMBS WITH RINGS AND BURDIZZO.

4.1 CHAPTER SUMMARY

The effects on plasma cortisol responses caused by using a burdizzo on the tail and scrotum in combination with a ring to castrate and tail dock were examined. Three different methods were examined. These were; ring only castration and tailing, burdizzo plus ring castration and tailing, and burdizzo plus ring castration and ring only tailing. It was found that the effect of using the burdizzo on the tail was negligible. It was also found that using the burdizzo in the conventional manner (one crush to each spermatic cord with no overlap of the 'cuts') on the scrotum and in the less conventional manner on the tail after ring application did not alter the cortisol response to ring castration and tailing. It is concluded that the most likely reason for the burdizzo not reducing the cortisol response to ring castration and tailing which has been demonstrated elsewhere (Kent *et al.* 1993, 1995) were the areas (anterior and posterior) of uncrushed tissue between the two burdizzo 'cuts' which would have allowed nociception from ischaemic scrotal tissue to be transmitted via undamaged neural fibres. Other possible reasons include differing burdizzo instruments, and size of lambs.

4.2 INTRODUCTION

The concept of using both a burdizzo and elastrator ring for lamb castration resulted from the hypothesis that disabling the afferent nerves contained in the spermatic cord by a burdizzo crush would prevent or substantially reduce transmission of nociception from ischaemic tissue distal to the crush and ring (Kent *et. al.*, 1993).

The hypothesis was developed on the following basis: Distress associated with ring castration as measured by cortisol and behavioural responses, can persist

for about 1.5-2.0 hours after treatment in lambs 1 week old (Mellor *et al.* 1991; Kent *et al.* 1993; Molony *et al.* 1993) and for about 3-3.5 hours in lambs 4-5 weeks old (Lester *et al.* 1991a; Kent *et al.* 1993; Molony *et al.* 1993). It has also been shown that afferent activity in the superior spermatic nerve resulting from ring application, persisted for in excess of 90 minutes in the lamb (Cottrell and Molony, in press), and nociceptor activity can continue for 3 hours after occlusion of the blood supply to the testes in the rat (Grubb *et al.* 1990). This nociception is presumed, at least partly, to stimulate plasma cortisol responses in lambs, responses which reach maximum values 60-90 minutes after application of a rubber ring (Lester *et al.* 1991a; Mellor *et al.* 1991; Kent *et al.* 1993; Molony *et al.* 1993). The burdizzo, used in conjunction with the elastrator ring reduced the cortisol response associated with ring castration and tailing in the trials of Molony *et al.* (1993) and Kent *et al.* (1993,1995) but not in the experiment described in Chapter 3 which involved castration alone.

These differences in the effects of using a burdizzo and ring for castration on the cortisol responses, may be due to a number of things.

1. The timing and length of burdizzo application. In the work described in Chapter 3 a burdizzo was used before the ring was applied as close as possible to the burdizzo 'cuts'. In contrast, Kent *et al.* (1993,1995) applied a burdizzo just distal to, and after application of a ring. This difference is not likely to result in the different cortisol responses but cannot be ruled out (see discussion). Also different was the duration of burdizzo application. In the present work (Chapter 3) a burdizzo was applied for 1, 5 or 10 seconds. In the work described by Kent *et al.* (1993), a burdizzo was applied for 6 seconds and in that described by Kent *et al.* (1995) application was for 10 seconds. These differences are not likely to have resulted in the differing cortisol responses, but cannot be ruled out.

2. The method of burdizzo application. Kent *et al.* (1993, 1995) applied the burdizzo once only across the whole width of the scrotum, distal to and after application of the rubber ring. In the work described in Chapter 3 the burdizzo was applied to each spermatic cord and the associated scrotal

tissue in the traditional manner. Care was taken to leave an area of undamaged tissue between each 'cut' to prevent necrosis of scrotal tissue. This area of undamaged tissue may, however, allow transmission of nociception from ischaemic scrotal tissue to the CNS through undamaged afferent nerve fibres.

3. Tailing possibly affected the outcome. The addition of tailing to castration using rings plus burdizzo may have confounded the comparison of the cortisol responses to the two ring plus burdizzo methods of castration. Kent *et al.*, (1993,1995) applied the burdizzo to the tail making no effort to apply the burdizzo between two tail vertebrae. Therefore it seems likely that in at least some lambs, bone will have been crushed by the burdizzo, which may result in more or less nociceptor activity than if the spinal cord was crushed between vertebrae.

4. Different burdizzo dimensions.

5. Different sized lambs.

In an attempt to clarify the results of the studies discussed above, a further trial was undertaken to answer the following questions.

1. Does the application of a burdizzo to the spermatic cords and the surrounding scrotal tissue, and to the tail, before ring castration and tailing affect the cortisol response to ring castration and tailing?

2. Does using a burdizzo on the tail affect the plasma cortisol response to ring tail docking combined with ring + burdizzo castration?

3. Can it be determined, by reference to the study described in Chapter 3, whether the timing of burdizzo use (before or after ring application) affects the cortisol response to burdizzo + ring castration?

4. Can it be determined, by reference to the results obtained by Kent *et al.* (1993), whether the manner in which the burdizzo is used, either across the

whole width of the scrotum or applied separately to each spermatic cord, affects the cortisol response to ring + burdizzo castration?

4.3 MATERIALS AND METHODS

4.3.1 Animals

Thirty Romney lambs aged between 45 and 55 days (mean 50 days), weighing between 11 and 25 kg (mean 17.9 kg), were castrated and tail docked using three different methods on a Massey University farm. There were 10 lambs in each of the three groups.

After the ram lambs had been weighed and their necks clipped to facilitate jugular venipuncture, ewes and their lambs were penned together (pen size 1.5 m by 2 m) 15 hours (overnight) before the trial began. Lambs' heads were marked with a scourable marker spray to allow easy identification. Ewe lambs of mixed sex twins were kept with the dam and ram twin. Approximately 1 hr before the start of the blood sampling, the lambs (including any ewe lambs) were separated from their dams by a wire mesh fence allowing them to see, smell, and hear their dams but not to feed.

Blood samples (7 ml) were taken by jugular venipuncture immediately prior to treatment and at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 minutes after treatment. In each case, this procedure usually took less than 15 seconds to complete.

4.3.2 Plasma cortisol assays.

Blood samples were collected into heparinised vacutainers, centrifuged immediately and then chilled until they were able to be frozen for storage at -20 °C.

Plasma cortisol concentrations were determined using a non-extraction radio-immunoassay (described in Chapter 3) with a lowest detectable concentration

of 1.5 nmol/l. The inter-assay and intra-assay coefficients of variation were 13% and 9.0%, respectively.

4.3.3 Treatments.

Ring Castration and Tailing (Ring CT).

A constricting rubber ring (Allflex New Zealand Ltd., Palmerston North) was applied to the neck of the scrotum using an elastrator (Elastrator Ltd; Blenheim; Fig. 3.2) after ensuring that both testes were distal to the ring. A ring was also applied to the tail at the junction of the caudal fold and the tail.

Ring + Burdizzo 6 Seconds Castration and Tailing (Ring + Burd 6 CT)

A rubber ring was applied to the scrotum as above. Following ring application, a small burdizzo clamp (The Ritchey Nipper, Ritchey Tagg Ltd. Masham, Nth Yorkshire, England. Fig 3.2 and Fig 3.3) was applied for 6 seconds to each spermatic cord and associated scrotal tissue just distal to the rubber ring. In addition the same burdizzo clamp was used on the tail immediately distal to the ring. No effort was made to position the burdizzo between two vertebrae.

Ring + Burdizzo 6 Seconds Castration and Ring Tailing (Ring + Burd 6 C & Ring T)

The same method of castration that was used for Ring + Burd 6 CT lambs was used in this group. However, lambs in this group were tailed with a rubber ring only.

4.3.3 Presentation of results

The cortisol concentrations for each group have been presented as the mean ± the standard error of the mean (SEM) at each sample time. The differences between the means at each time point were analysed using two way analysis of variance (ANOVA) and Student's t test.

To correct for individual variation, the pre-treatment value was subtracted from the concentration in every subsequent sample. Graphical presentation is therefore expressed in terms of change in the mean plasma cortisol concentrations from the values at 15 minutes before treatment (t = -15 minutes).

All cortisol values are presented in Appendix 2.

4.4 RESULTS

Pre-treatment cortisol concentrations (mean ± SEM) are presented in Fig. 4.1. There were no significant differences between the groups.

The mean plasma cortisol response for each group has been illustrated in Fig 4.2. There was a significant increase in plasma cortisol concentration after treatment in each group ($p<0.001$). Maximum values were reached 30 at minutes after treatment for Ring + Burd 6 C & Ring T lambs and at 45 minutes after treatment for Ring + Burd 6 CT and Ring CT lambs. Cortisol concentrations were significantly elevated for 2 hours after treatment and had

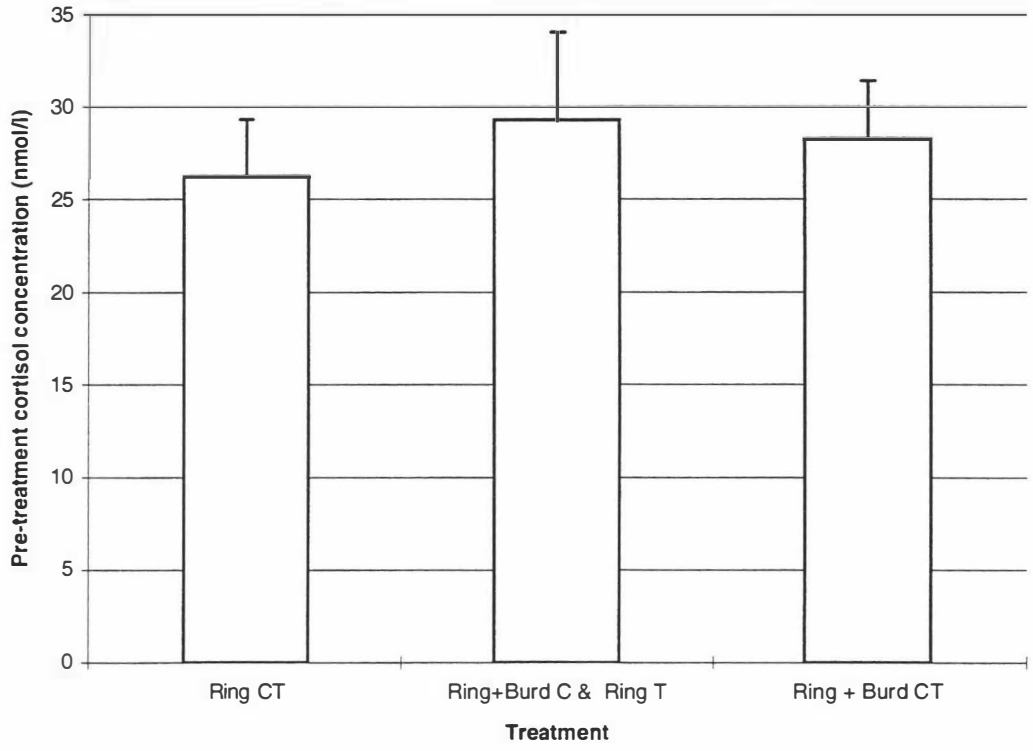


Fig 4.1: Pre-treatment cortisol concentrations (Mean ± SEM) for castration plus tailing study.

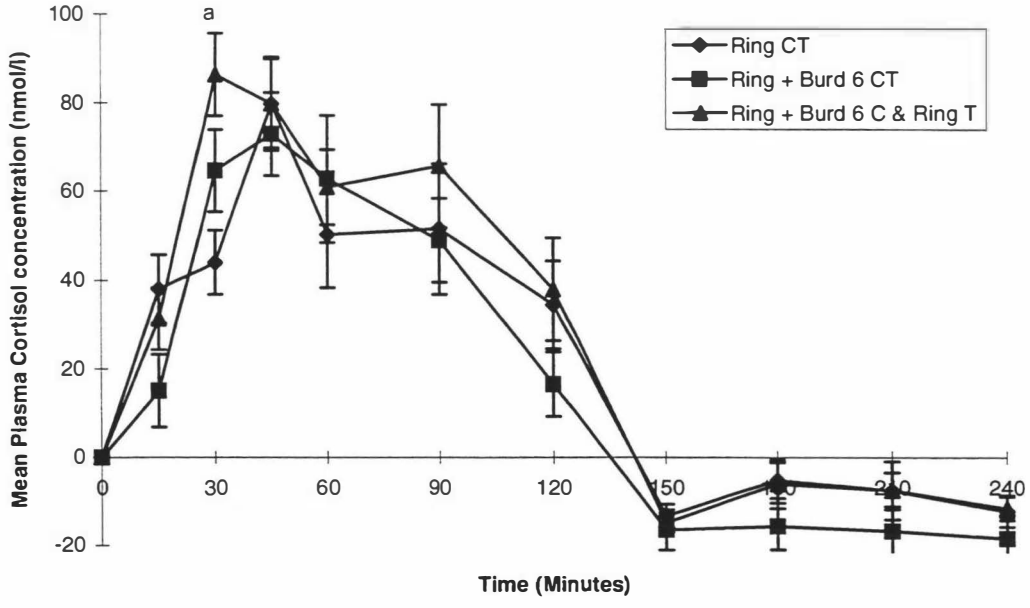


Fig 4.2 Change in plasma cortisol concentrations (mean ± SEM) in lambs castrated and tailed using different methods. Superscript (a) indicates significant difference ($p<0.05$) from Ring CT.

returned to or below pre-treatment values 3 hours after treatment in all groups. The mean cortisol concentration of Ring + Burd 6 C & Ring T lambs at 30 minutes after treatment was significantly higher than the corresponding mean concentration of Ring CT lambs. There were no other significant between-group differences.

4.5 DISCUSSION

The peak concentrations in this trial were less than those reported by Kent *et. al.* (1993) for 42 day old lambs, but similar to those reported by Lester (1991) and Mellor and Murray (1989a). The difference may have been due to the breed used, the age of the lambs or the assay method.

The results of this study suggest that there is no advantage in using a burdizzo in combination with rubber rings in the way described, to decrease the magnitude or duration of the cortisol response to ring castration plus tailing. This supports the findings in Chapter 3 which suggest that there is no advantage in using the burdizzo in the way described to reduce the cortisol response to ring castration alone. However, these results differ from those obtained by Kent *et al.* (1993, 1995).

It is likely that the way in which the burdizzo was applied when used in combination with a ring for castration affected the magnitude and duration of the cortisol response. Kent *et al.* (1993,1995) found that a reduction in the magnitude and duration of the cortisol response to ring castration and tailing was accomplished when using a burdizzo in a single application across the whole width of the scrotum and tail. This would have crushed both the spermatic cords and scrotal tissue in one action and the tail in the other. The crush on the scrotum is likely to have damaged all afferent nerve fibres within the scrotum and spermatic cords that pass through the crushed tissues. This is likely to have prevented or markedly reduced transmission of nociception from the scrotum and testes distal to the crush. The methods of burdizzo application

used in the present study and that described in Chapter 3 were likely to have sufficiently damaged the spermatic nerves to prevent transmission of nociception from the ischaemic testes. As discussed in Chapter 3, however, they may not have completely prevented nociception from ischaemic scrotal tissue because an area of undamaged tissue remained between the two burdizzo 'cuts' through which afferent nerves will pass. Because the method of application of the burdizzo is the only major difference between the present study and that described by Kent *et al*. (1993, 1995), it may be proposed that the cortisol responses were different for this reason. If so, the noxious input from the scrotum using the present method was apparently sufficient to overcome the beneficial effects of disabling the spermatic nerves.

It is unlikely that the timing of the use of the burdizzo, either before or after ring application, will have any effect on the cortisol response to ring plus burdizzo castration. This is because the immediate effect of burdizzo application which is thought to be a marked barrage of nociception from damaged afferent nerve fibres, will not be prevented by ring application. It has been shown that nociception from ischaemic testicular tissue transmitted through afferent nerve fibres is not prevented by the ring (Grubb *et al*. 1990, Cottrell and Molony, in press). The immediate effect of ring application is thought to be minor and the nociception (the transmission of which is intended to be prevented by the burdizzo) from ischaemic tissue distal to the ring is slow to develop (5-15 minutes). Therefore, the use of the burdizzo before or immediately after ring application is not likely to make any difference to the cortisol response. This is supported by the results from Chapter 3 in which the burdizzo was applied before ring application, when compared to the results from the present study in which the burdizzo was applied immediately after ring application. Both studies suggest that there was no detectable benefit in using the burdizzo at all to reduce the cortisol response to ring castration with or without tailing.

The duration of burdizzo application in the manner described in this study and Chapter 3, is not likely to have resulted in the failure to successfully reduce the magnitude or duration of the cortisol response to castration with or without tailing with rubber rings. This is because in the study described in Chapter 3,

there were no major differences between the magnitudes and durations of the cortisol responses of lambs castrated with a burdizzo, which was applied for 1, 5 or 10 seconds, plus subsequent ring application. In the present study and the study described in Chapter 3, the burdizzo was applied twice and hence the burdizzo was actually applied for a total of 4,10, or 20 seconds to each lamb rather than once for 6 or 10 seconds as by Kent *et al.* (1993,1995). The total duration of burdizzo application probably did not influence the cortisol response, but the fact that two different applications, and hence two tissue insults, occurred in this study and that described in Chapter 3 may have influenced the cortisol response. However, as it is likely that there is little difference in the number of nerves crushed, or indeed less nerves crushed when applying the burdizzo twice, it does not seem probable that this is a viable explanation.

Another difference between the studies discussed in this thesis (present Chapter and Chapter 3), and those described by Kent *et al.* (1993,1995) is the type of burdizzo used. Design differences can include jaw dimensions, gap between closed jaws and the pressure exerted with each crush. Although these could have had a significant effect on the results of both studies, the design of the present study does not permit the assessment of these effects on the cortisol responses.

There was a difference in duration of the cortisol responses in the present study and those in the study described in Chapter 3. The duration of the cortisol responses of lambs castrated with rings only or with a burdizzo plus rings was approximately 180 minutes (Chapter 3), whereas the response duration in the present study was approximately 150 minutes. The difference in durations may be a result of differences in lambs, situations and pre-treatment values.

It was anticipated that as tailing alone using a rubber ring causes comparatively low levels of distress (Lester *et al.* 1991a), the use of the burdizzo on the tail, during the ring plus burdizzo castration and tailing procedure, was not likely to further reduce the pain and would therefore make the procedure unnecessarily complicated. The present results seem to support this view, as there were no

significant differences between the cortisol responses of Ring + Burd 6 C & Ring T lambs and Ring + Burd 6 CT lambs. However, it must be noted that the greater of two noxious inputs (ie from castration and tail docking) can apparently influence the magnitude and duration of cortisol responses to these procedures (Lester *et al.* 1991a). Therefore, the results from this study suggest one or both of two possible outcomes of using the burdizzo on the tail.

1. Using the burdizzo on the tail as well as a ring did not increase the noxious input from the tail of lambs castrated with a rubber ring and burdizzo as judged by the cortisol response. To clarify this point, the cortisol responses of lambs which had been tailed only using a ring or a ring + burdizzo would need to be compared.

2. Castration with a burdizzo and ring was more noxious than tailing with a ring or with a ring + burdizzo. This is likely as castration with a ring has been shown to be more noxious than tailing with a ring and as noxious (eliciting a similar cortisol response) as castration and tailing with a ring (Lester *et al.* 1991a).

Using the burdizzo on the tail, with no effort made to apply the burdizzo between two vertebrae, is likely to crush bone in some lambs. This may explain the two groups of responders described by Kent *et al.* (1993). Some lambs castrated and tailed with a ring plus burdizzo exhibited cortisol responses that were greater in magnitude and longer in duration than the other lambs. It is possible that the greater responses were elicited by a vertebra being crushed by the burdizzo. This may have resulted in the noxiousness of tailing being of sufficient intensity to influence the magnitude and duration of the cortisol response to both castration and tailing.

The following conclusions can be drawn from the present results.

1. The burdizzo, used to crush each spermatic cord and associated tissue separately as well as the tail, did not successfully reduce the cortisol response to ring castration and tailing.

2. The cortisol response caused by castration and tailing with the burdizzo plus ring was either mainly influenced by the noxious input caused by castration rather than tailing, or the use of the burdizzo on the tail did not add to the effect of ring application. However, the results from this study cannot determine which is the case.

3. Applying the burdizzo before or after ring application is not likely to alter the cortisol response to ring plus burdizzo castration and tailing.

4. The duration of burdizzo application when used in the way described in this study and the study described in Chapter 3, is not likely to affect the cortisol response to ring castration or tailing.

5. It is possible that for the burdizzo to be successful in reducing the pain-induced distress (as indicated by cortisol responses) resulting from castration with a rubber ring, the burdizzo must be applied across the entire width of the scrotum, as described in Kent *et al.* (1995).

CHAPTER 5: BEHAVIOURAL ASSESSMENT OF PAIN AND/OR DISTRESS CAUSED BY CASTRATION.

5.1 CHAPTER SUMMARY

The behavioural responses to different castration methods were observed for four hours after treatment. The methods of castration used were ring, burdizzo plus ring and burdizzo. Short scrotum creation was also studied, as were the effects of using local anaesthetic prior to treatment. For each method of castration, whether or not the particular behaviours were elicited by sensory input from the immediate area was tested by observing the effects of prior local anaesthetic administration. If local anaesthetic administration prior to castration elicited behaviours at levels similar to those exhibited by Controls, it was assumed that the particular behaviour was indeed a result of modified sensory input caused by the method of castration. It was found that many behaviours were not at levels similar to controls after local anaesthetic administration, suggesting that either local anaesthetic was not effective or that something other than sensory input from the area was responsible for the behaviour. Behaviours exhibited after ring castration that were returned to Control levels by local anaesthetic administration were restlessness and lateral recumbency. Ventro-lateral recumbency, although significantly reduced by using local anaesthetic may have been partly caused by something other than sensory input from the immediate area. Residual sensory input, at a level that does not stimulate restlessness, may also be the reason for incomplete removal of ventro-lateral recumbency. This would indicate that ventro-lateral recumbency is a more sensitive measure of sensory input than cortisol or restlessness after ring castration. This raises the question of whether or not the residual sensory input was likely to be noxious and of any consequence in welfare terms. Burdizzo plus ring castration led to similar behavioural responses as in Ring lambs, although restlessness was the only behaviour at levels similar to Controls after local anaesthetic administration. Although burdizzo castration significantly reduced the amount of normal walking or standing, no one specific abnormal behaviour was significantly higher than in Controls. Local anaesthetic

had no effect on any behavioural parameter after burdizzo castration. Although this may suggest that little or no change in sensory input was caused by burdizzo castration this seems unlikely. Due to the different natures of the behavioural responses of burdizzo lambs and ring lambs it is believed that it is not possible to compare the intensities of pain-induced distress using behaviour. As ring and burdizzo plus ring, which are similar treatments, also have behavioural responses in common it was thought to be appropriate to compare the intensities of pain-induced distress, but no significant differences were found.

5.1 INTRODUCTION

There is no one reliable parameter for assessing the pain-induced distress experienced by an animal (Stafford and Mellor 1993). It is therefore desirable to evaluate more than one parameter to assess distress (Dawkins 1980; Levine 1985; Broom 1993).

When an animal experiences pain or discomfort it will behave to remove or minimise the pain or discomfort experienced. The behaviour expressed by an individual will depend on the species of animal, the environmental conditions, the source and intensity of the pain or discomfort experienced, and the individual temperament of that animal. Although there are many influences on the behavioural response, under controlled conditions and with an understanding of the limitations, attempts have been made to use behaviour to assess the duration and intensity of pain-induced distress (Mellor and Murray 1989a,b; Lester *et al.* 1996).

Studies of calf behaviour after castration or tail docking have proved to be useful in assessing whether or not the animal was experiencing pain-induced distress (Macauley and Friend, 1987; Mellor *et al. 1*991; Robertson *et al.* 1994; Petrie *et al.* 1995). If the behavioural changes are correlated with a significant cortisol response then it is likely that the pain or discomfort caused by the treatment is responsible for those behavioural changes. Macauley and Friend

(1987) found a correlation between high levels of plasma cortisol and observed incidences of standing, kicking and cantering in response to castration. Lay *et al.* (1992) have shown that either freeze or hot-iron branding causes an increase in plasma cortisol concentrations and greater 'escape avoidance' reactions in branded cows compared to controls. Dehorning by different methods which are known to elicit large cortisol responses (Sylvester *et al.* 1993), resulted in behaviours such as head shaking, footstamping and tail flicks (Sylvester S.P, Mellor D.J., Stafford K.J, Bruce R.A., and Ward R.N., unpublished data).

Surgical or ring castration plus tail docking caused behavioural changes in lambs that may have indicated distress. This was probably due to pain arising from increased nociceptor activity in the scrotum, testes, and/or tail (Mellor and Murray 1989a,b; Mellor *et al.* 1991; Molony *et al.* 1993; Kent *et al.* 1995; Lester *et al.* 1996). Plasma cortisol concentrations were also used in these studies to ascertain the relative intensities and durations of distress experienced by these animals (Mellor and Murray 1989a,b; Mellor *et al.* 1991; Lester *et al.* 1991a,b; Molony *et al.* 1993; Kent *et al.* 1995).

When behaviours are used as indicators of distress, it is important not to underestimate the importance of immobility compared to more obvious increases in activity. Underestimating the importance of different behavioural responses can lead to erroneous conclusions about which treatment causes more distress (Stafford and Mellor, 1993). For example, castration and tail docking with rubber rings elicited characteristic behaviours that were rarely exhibited by lambs treated in any other way. These behaviours included lateral recumbency, writhing, kicking, and increased restlessness (Mellor and Murray 1989a,b; Mellor *et al.* 1991, Lester 1991, Molony *et al.* 1993; Kent *et al.* 1995; Lester *et al.* 1996). Surgical castration and tail docking did not elicit such an active and easily distinguishable behavioural response. However, more instances of abnormal standing, especially statue standing and legs splayed and walking with tentative steps, have been noted in surgically castrated and tailed lambs (Molony *et al.* 1993; Lester *et al.* 1996;). The difference between behavioural responses has led to the conclusion being made that surgical

castration and tailing is less distressful than ring castration and tailing (Shutt *et al.* 1988). This conclusion has been disputed by Mellor and Holmes (1988), Barnett (1988) and Lester *et al.* (1991a,b) and more recently by Mellor and Molony (1995) and Lester *et al.* (1996). Plasma cortisol responses indicated that surgical castration and tailing elicited a greater intensity of noxious input for a longer duration than ring castration and tailing (Lester *et al.* 1991a; Kent *et al.* 1993).

At the time this study was planned and carried out the behavioural response to burdizzo castration had not been characterised. Kent *et al.* (1995) have since reported that burdizzo castration and tailing resulted in behaviours such as abnormal ventral lying and abnormal standing (especially statue standing), that were evident for 90 minutes after treatment. When lying down, burdizzo treated lambs were not as active as ring castrated lambs, and sometimes adopted a dog sitting posture (Kent *et al.* 1995).

Burdizzo + ring castration and tailing has been shown to result in less active behaviours than ring castration and tailing (Molony *et al.* 1993). Fewer instances of abnormal lying, and abnormal standing were seen in burdizzo + ring lambs than in ring lambs (Molony *et al.* 1993).

The present study was designed to;

- Characterise the behavioural responses to burdizzo castration, burdizzo + ring castration and short scrotum creation without the influences of tail docking, and compare them to the known responses of ring castration.
- Assess the validity of using particular behavioural parameters to assess the relative pain-induced distress caused by each treatment.
- Assess the relative pain-induced distress experienced after ring, burdizzo + ring, or burdizzo castration.

- Assess the effect of local anaesthetic, after it was injected into different sites, on the behavioural response to ring, burdizzo + ring, or burdizzo castration.

5.2 MATERIALS AND METHODS

5.2.1 Animals

The same lambs that were used in Chapter 3 were used for this behaviour study. The present study and the study described in Chapter 3 were performed concurrently.

5.2.2 Observations

One observer recorded the behaviours of two lambs in a single pen. The observer was ignorant of the treatment that each lamb had received. The lambs were observed continuously for 1 hour after treatment and the behaviour and/or posture were recorded every 15 seconds. Between 1 and 2 hours after treatment, lambs were observed for 1 minute at 15 minute intervals. Behaviour and/or posture were recorded every 15 seconds throughout the minute of observation. Between 2 and 4 hours after treatment, lambs were observed for 1 minute at 30 minute intervals. Again, behaviour and/or posture were recorded every 15 seconds throughout the minute of observation.

Nine individual observers participated throughout the trial. Prior training included; a) video observation of surgically or ring castrated and tailed lambs and control lambs. b) simultaneous observation, recording and subsequent evaluation of behaviour in a pilot study involving Burdizzo, and ring + burdizzo lambs.

Behaviours that were observed and recorded were;

- **Normal Standing/Walking**. A lamb was deemed to be standing or walking normally if both the sternum and abdomen were off the ground and the lamb was not exhibiting any abnormal standing/walking behaviours described below.

- **Legs Splayed.** A lamb with it's hind legs splayed apart greater than 5 cm either side of the pelvic bone.

- **Tail Cocked.** A lamb with it's tail lifted away from its anus

- **Head against Rails.** A lamb pushing the top of its head against the rails of the pen.

- **Statue Standing.** A lamb that was standing motionlessly, seemingly unaware of it's surrounding environment (Molony *et al.* 1993).

- **Arched Back.** A lamb with a hunched rounded back.

- **Legs Extended.** A lamb standing with it's hind legs pushed out posteriorly such that the bottom of the tail was in vertical alignment with (between the legs), or anterior to the hind hooves.

- **Staggering.** A lamb walking with some abnormality of gait indicated by stiffness, swaying, stamping of the legs or ataxia.

- **Pacing.** A lamb that walks around the pen constantly, seemingly without an objective.

- **Stand Kick.** A lamb that kicks with it's hind limb either forward or backwards whilst standing.

- **Ventral Recumbency.** A lamb lying down with both front and rear legs tucked under the sternum and abdomen.

- **Ventro-lateral Recumbency.** A lamb lying down with the front legs tucked under the sternum but the hind legs extended posteriorly or to one side. The hind legs were considered extended if one leg was fully visible and the underlying leg was visible from the hoof to the hock.

- **Lateral Recumbency.** A lamb lying down on its side with both the front and rear legs extended.

- **Recumbent Kick.** A lamb lying down in any of the recumbent positions that kicked with one or both of it's hind legs.

- **Restlessness** was scored as the number of times that a lamb stood up or lay down, with 1 point awarded each time it stood up and 1 point each time it lay down (Mellor and Murray,1989a: Lester *et al.* 1996).

5.2.3 Presentation of Results

Where appropriate the results are presented as the mean ± the standard error of the mean (SEM).

Restlessness has been calculated as the mean ± SEM for five minute intervals during the first hour after treatment. The total amount of restlessness (mean ± SEM), ie the number of times an animal stood up or lay down in the first hour after treatment, has been presented.

The percentage of observations (mean ± SEM) was calculated for each 15 minute period during the first hour, for each 30 minute period for the next two hours and for the final hour. For example, in the first 15 minutes after treatment, one lamb's behaviour was recorded 60 times. If there were 30 recordings of ventral lateral recumbency, 15 of normal standing/walking and 15 of abnormal standing/walking behaviour, then this would be presented as 50%, 25% and 25% of observations, respectively.

Also presented is the percentage of all behaviours observed during the first hour after treatment and for 4 hours after treatment. For example, if ventro-lateral recumbency was observed 130 times out of a possible 260 observations during four hours, this would be presented as 50%.

Comparisons between the mean percentage of behaviours observed after different treatments were made using Students t-test (Microsoft Excel v 5.0). A parametric test was used in accordance with similar past research comparing behaviours after different treatments (Mellor *et al.* 1991; Molony *et al* 1993, Robertson *et al* 1994; Lester *et al.*1996). However, in the late stages of the preparation of this thesis it became evident that a non-parametric statistical test could also have been used, and may have been more appropriate, to make these comparisons (Martin and Bateson 1990). Due to time constraints on completion of this thesis, these were not carried out, but before any publications are submitted non-parametric tests will be completed and the results compared to the those obtained using the parametric tests. It is not anticipated that any major differences will be noted.

In view of the large number of figures, the most important figures are located at the end of the results section, and the rest including tables of statistical comparisons, are in appendix 3.

5.4 RESULTS

Legs Splayed, Legs Extended, Tail Cocked, Head Against Rails, Statue Standing, Backwards Walking, Pacing, Standing Kick.

Very few (0-3% of behaviours exhibited in each time period) of these behaviours or postures were exhibited by lambs in any group. Because of the low number of observations of each of these behaviours, all these observations excluding 'head against rails' were pooled and called 'abnormal standing/walking' which provided a more useful behavioural measure. The presence of these behaviours in control lambs indicated the insensitivity of

these measurements. The 'head against rails' posture was not observed throughout the study and so was excluded from all analyses.

Controls

With two exceptions, there were no significant differences in any behavioural parameter between any of the Control and LA Control lambs. The exceptions were significant differences ($P<0.05$) between total amounts of normal standing/walking exhibited by LA CD Control and LA SC Control lambs, and LA CD Control and LA SC + CD Control lambs during the 4 hours of observation (Appendix 3, Table 2). Control and LA Control lambs predominantly exhibited normal standing/walking for 4 hours after treatment (Appendix 3, Fig. 1). In addition, lambs would lie down in a ventral position from 2 - 4 hours after treatment (Appendix 3, Fig. 2). A small amount (<10%) of abnormal standing/walking was exhibited during the four hours after treatment (Fig. 5.4). Restlessness was not a feature of the behaviour exhibited by Control or LA Control lambs in the first hour after treatment. The highest mean restlessness score exhibited by a control group was 5 (Fig. 5.17). LA SC lambs displayed a small amount of abnormal recumbency, however due to the large standard error this was not significantly different from the other controls which exhibited none.

Ring lambs

Ring lambs exhibited little normal standing/walking in the first hour after treatment (<20%) (Fig. 5.1; Appendix 3, Fig. 1). However, between 2 and 4 hours after treatment normal standing/walking was the predominant behaviour (Appendix 3, Fig. 1). In the first hour after treatment abnormal standing/walking was exhibited for a significant proportion of the time (15%) (Fig. 5.4), as was abnormal recumbent behaviour (Fig. 5.7) or more specifically ventro-lateral (Fig. 5.9) or lateral recumbency (Fig. 5.11). High restlessness was a major feature of the early behavioural response to Ring castration. Ring lambs exhibited one of the highest restlessness scores of any treatment (approx 75) (Fig. 5.17).

Ring + Local Anaesthetic.

Lambs that had local anaesthetic administered prior to ring application exhibited notable amounts of normal standing/walking in the first hour after treatment (40-50%) (Fig. 5.1, Appendix 3, Fig. 1). These amounts were significantly ($p<0.05$) more than that exhibited by Ring lambs, but were significantly less than in all controls (Appendix 3, Table 1). Ring LA lambs exhibited between 15 and 30 % abnormal recumbency for one and four hours after treatment (Figs. 5.8, 5.9). This was virtually all ventro-lateral recumbency as no significant amounts of lateral recumbency were observed (Figs 5.10, 5.12). The amount of ventro-lateral recumbency exhibited by Ring LA SC and Ring LA SC+CD lambs during the 4 hours after treatment was significantly ($p<0.05$) less than in Ring lambs but not in Ring LA CD and Ring LA T lambs(Fig. 5.10; Appendix 3, Table 10). These amounts were significantly ($p<0.05$) higher than Controls. Restlessness scores varied between 12 and 48 depending on the site of local anaesthetic administration, however all were significantly ($p<0.05$) less than in Ring lambs (Fig. 5.17). The lowest restlessness score was in Ring LA SC+CD and the highest was in Ring LA CD lambs.

Burdizzo + Ring

Lambs that were castrated with a burdizzo and a ring exhibited between 20-25% normal standing/walking in the first hour after treatment (Fig. 5.1). Between 10 and 20% of behaviour in the first hour was abnormal standing/walking (Fig. 5.3). Abnormal recumbent behaviours were prevalent in the first hour after treatment (Fig. 5.7). This was almost entirely ventro-lateral recumbency (25-45%) as only insignificant amounts of lateral recumbency were observed (Figs 5.9, 5.11). Restlessness scores were all approximately 45 (Fig. 5.17).

Burdizzo + Ring and Local Anaesthetic.

Lambs which had local anaesthetic administered prior to Burdizzo 10 + Ring castration exhibited significant amounts of normal standing/walking (50-55%) during the first hour (Fig. 5.1) and for 4 hours (Fig. 5.2) after treatment. These were significantly higher than Burd 10 + Ring castrated lambs and although numerically lower than controls, this difference was not significant. Ventro-lateral recumbency contributed between 10 and 15% of behaviour in the first hour and for 4 hours after treatment (Figs 5.9, 5.10). This was significantly ($p<0.05$) less than in Burd 10 + Ring lambs although significantly ($p<0.05$) higher than Controls (Appendix 3, Table 10). Abnormal standing/walking contributed between 12 and 15 % of behaviours in the first hour (Fig. 5.1). This was not significantly different from values in Burd 10 + Ring Lambs or indeed, in Controls. Restlessness scores were 12 and 25 depending on the site of local anaesthetic administration (Fig. 5.17). This was significantly ($p<0.05$) less than the restlessness score of Burd 10 + Ring lambs (Appendix 3, Table 17).

Burdizzo castration

The behaviour of lambs castrated with a burdizzo was characterised by significant amounts (30-45%) of abnormal standing/walking in the first 15-30 minutes after treatment (Appendix 3, Fig. 1). In the first hour abnormal standing/walking contributed between 20 and 25% of behaviour (Fig. 5.3). This abnormal standing was not predominantly one behaviour but statue standing was not recorded at all. Normal standing/walking contributed between 45 and 60% of behaviour in the first hour with the remaining behaviour being predominantly ventral recumbency (Fig. 5.1). Abnormal recumbency (ventro-lateral or lateral) was not prevalent or was absent (Figs 5.7, 5.9, 5.11). Restlessness was not a significant feature of the behavioural response to burdizzo castration as all restlessness scores were less than 10 (Fig. 5.17).

Burdizzo + Local anaesthetic.

Lambs that had local anaesthetic administrated before burdizzo (10 seconds) castration exhibited predominantly normal standing/walking behaviour (55-65%)

during the first hour and for 4 hours after treatment (Figs. 5.1,5.2). This was not significantly different from Burd 10 lambs or to Controls. Abnormal standing/walking contributed 15-25% of behaviours in the first hour and for 4 hours after treatment in Burd + LA lambs (Figs. 5.3,5.4). This was not significantly different from the values in Burd 10 lambs or indeed, in Controls. Abnormal recumbency was almost all ventro-lateral and made up to 15% of behaviours in the first hour after treatment (Fig. 5.9). The values were not significantly different from the amount exhibited by Burd 10 lambs. The amount of total abnormal behaviours exhibited by Burd 10 lambs was not affected by prior local anaesthetic administration (Figs. 5.15, 5.16) Restlessness was not a feature of Burdizzo + LA lambs behaviour (Fig. 5.17). As was the case in Burdizzo lambs all restlessness scores were less than 10.

Comparisons between treatments.

Ring - Controls

Ring castration caused significantly ($p<0.05$) more abnormal recumbency (lateral and ventro-lateral) than was seen in than Controls (Appendix 3, Table 9). This was reflected in significantly ($p<0.05$) less normal standing/walking behaviour being exhibited (Appendix 3, Table 2). Restlessness was significantly higher in Ring lambs than in Controls.

Ring - Burdizzo + Ring.

There were no significant differences in any behavioural parameter between Ring and Burdizzo + Ring lambs.

Ring - Burdizzo

Ring lambs exhibited significantly ($p<0.05$) more abnormal recumbent behaviours (ventro-lateral and lateral) than Burdizzo lambs (Appendix 3, Table 8). This was reflected in Ring lambs exhibiting significantly less normal standing/walking behaviour than Burdizzo lambs (Appendix 3, Table 2). There

was no significant difference in the amount of ventral recumbency or abnormal standing/walking. There was significantly less ($p<0.05$) restlessness in Burdizzo lambs than in Ring lambs (Appendix 3, Table 17).

Burdizzo - Burdizzo + Ring

There was significantly less ($p<0.05$) normal standing/walking in Burdizzo + Ring lambs than in Burd 1 lambs, but the difference was not significant between Burd 10 lambs and Burdizzo + Ring lambs (Appendix 3, Table 1). Burdizzo lambs exhibited significantly less ($p<0.05$) abnormal recumbency than Burdizzo + Ring lambs (Appendix 3, Table 8). Burdizzo lambs also exhibited significantly less ($p<0.05$) restlessness than Burdizzo + Ring lambs (Appendix 3, Table 17).

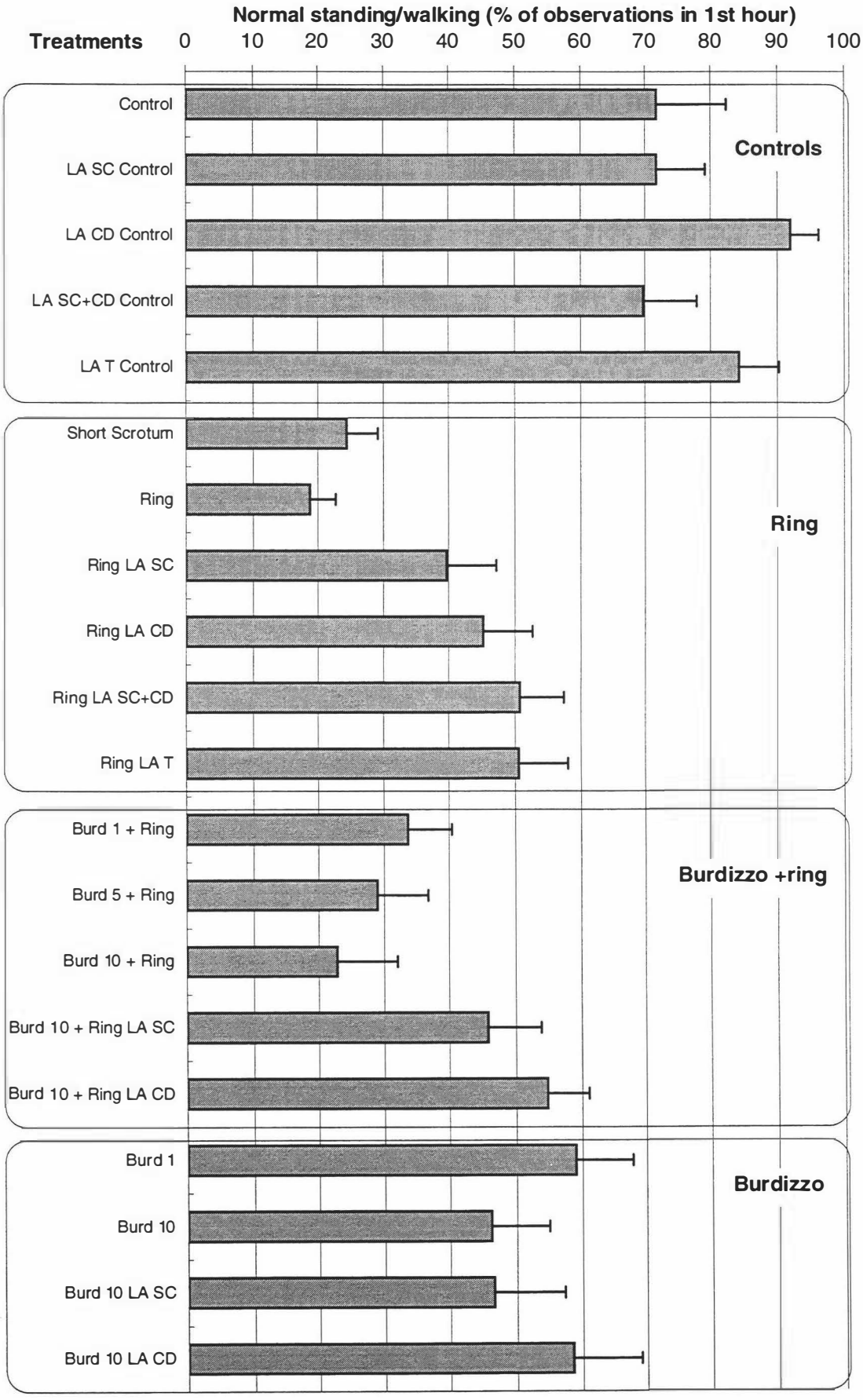


Fig 5.1 The percentage of behaviours (mean + SEM) observed in 60 minutes that were normal standing/walking. For significant differences see appendix 3, Table 1.

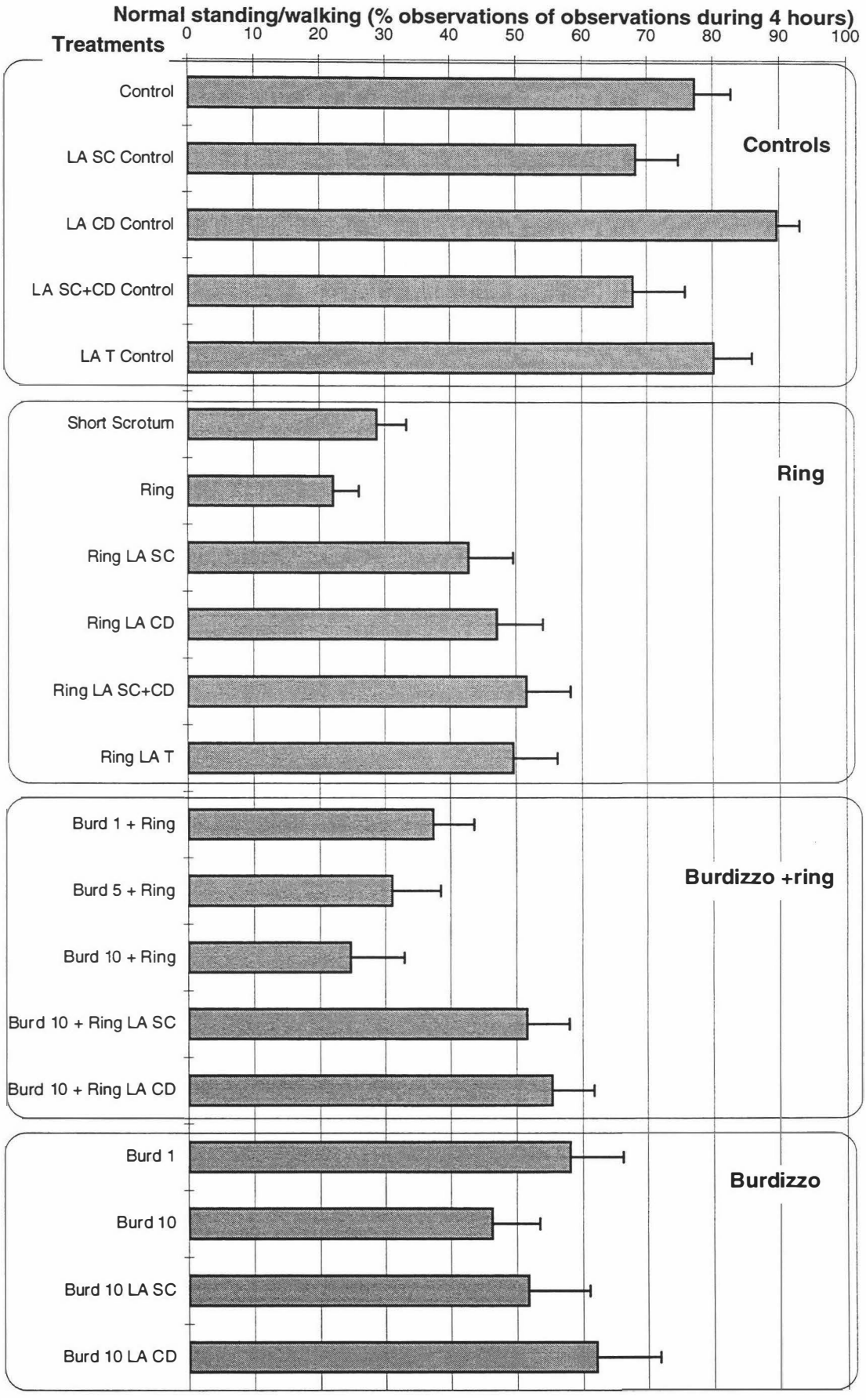


Fig 5.2 The percentage of behaviours (mean + SEM) observed in 4 hours that were normal standing/walking. For significant differences see appendix 3, Table 2.

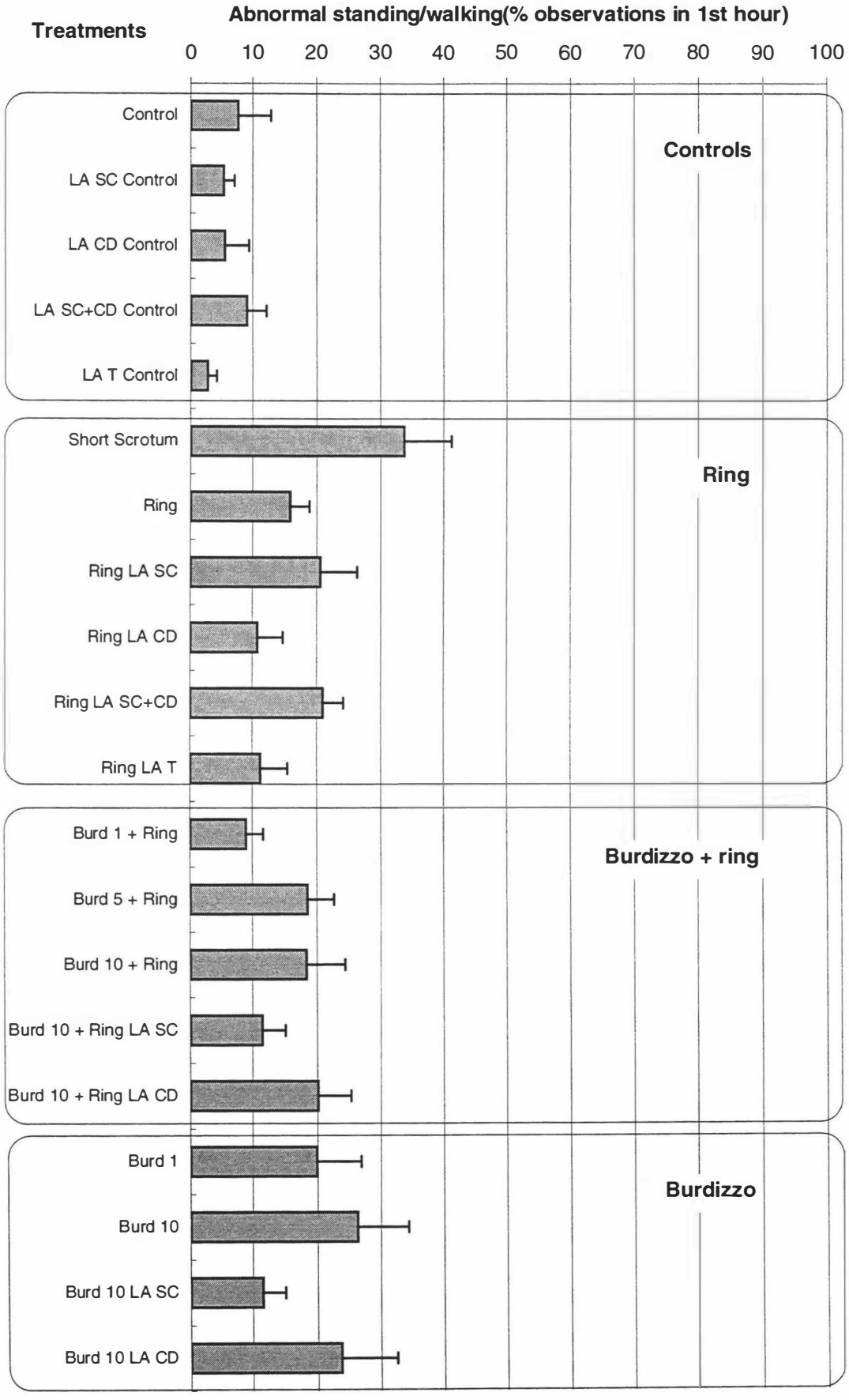


Fig 5.3 The percentage of behaviour (mean + SEM) observed in 60 minutes that was abnormal standing/walking. For significant differences see appendix 3, table 3.

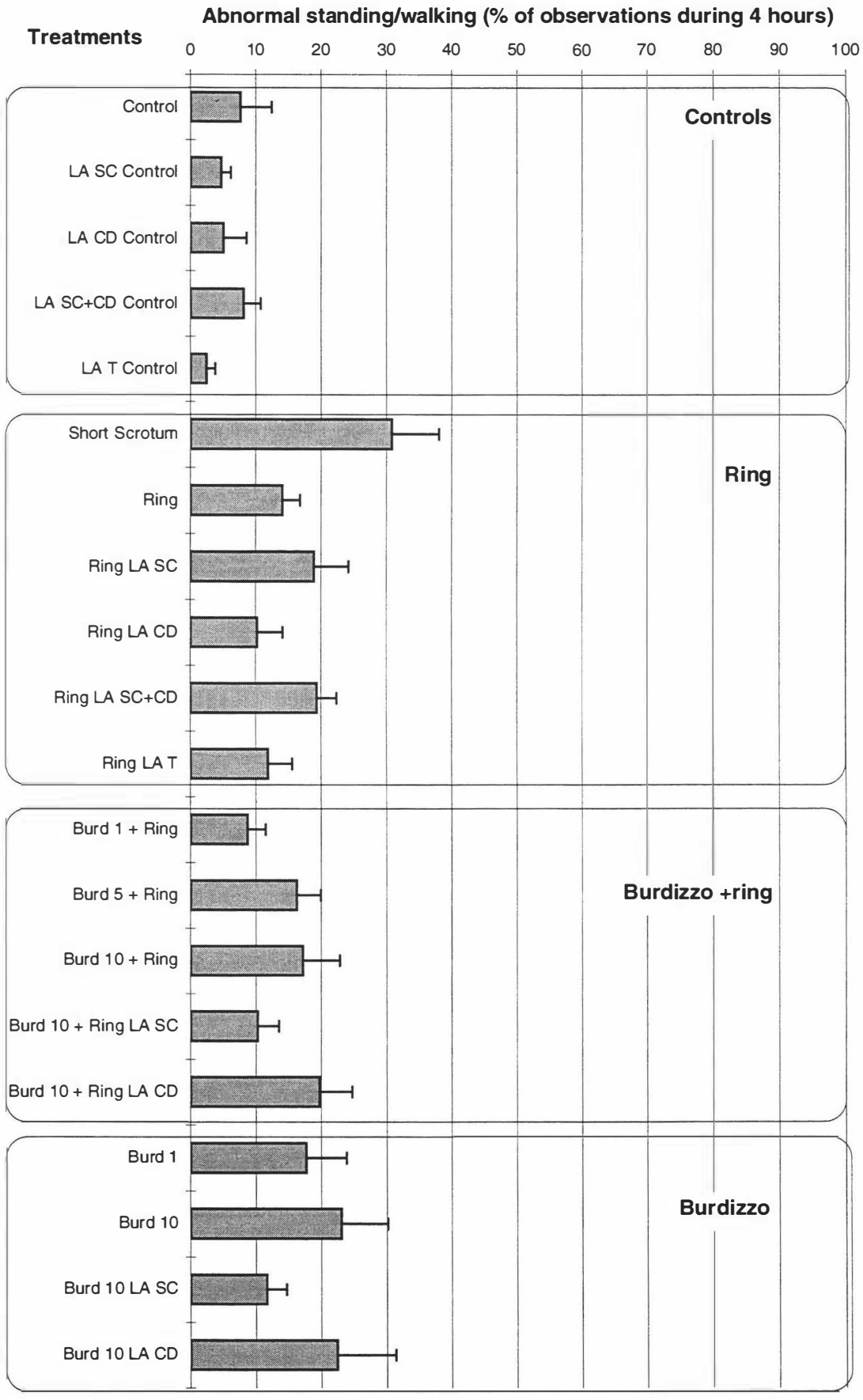


Fig 5.4 The percentage of behaviours (mean + SEM) observed in 4 hours that were abnormal standing/walking. For significant differences see appendix 3, Table 4.

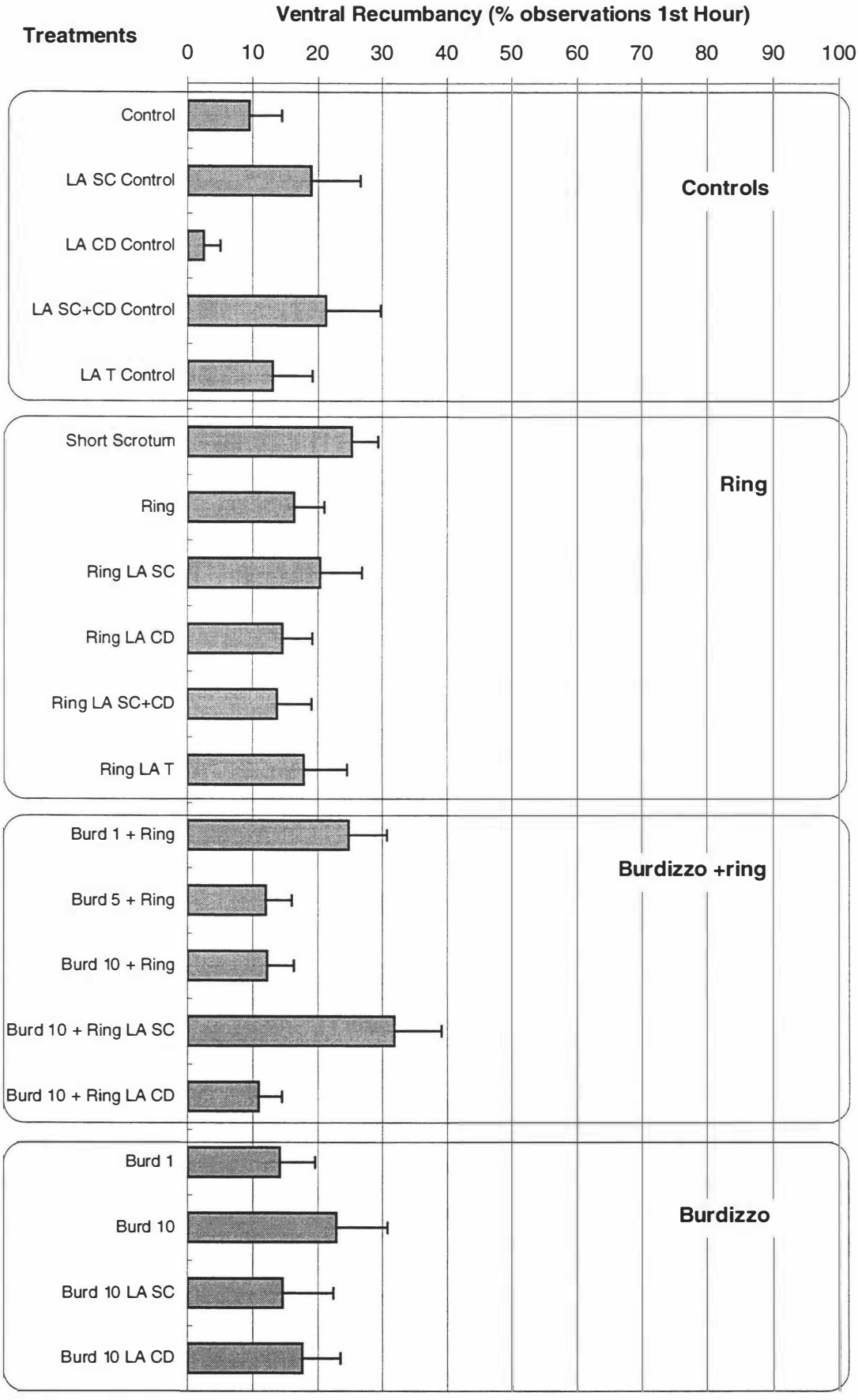


Fig 5.5 The percentage of behaviour (mean + SEM) observed in 60 minutes that was ventral recumbancy. For significant differences see appendix 3, Table 5.

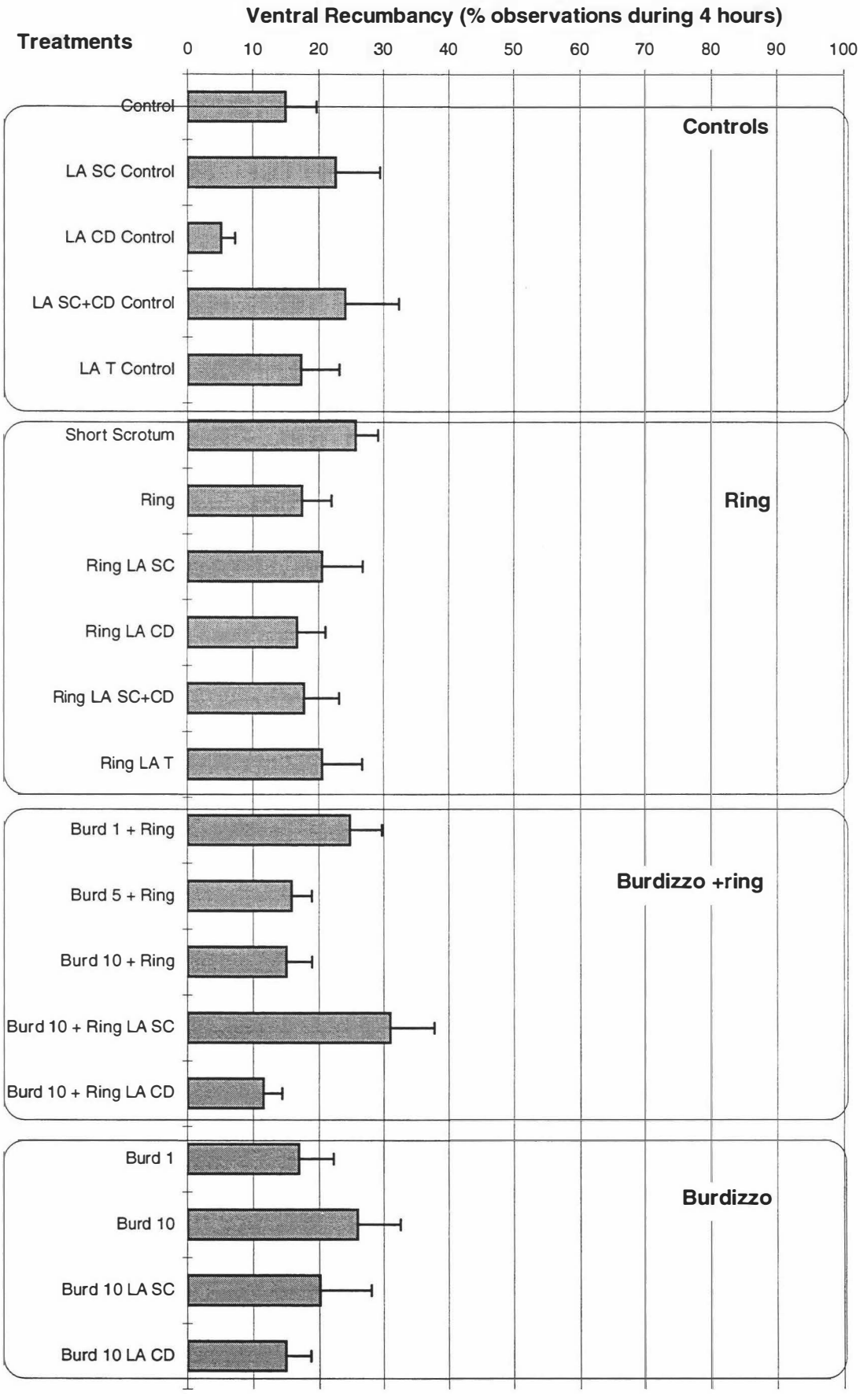


Fig 5.6 The percentage of behaviours (mean + SEM) in 4 hours that were ventral recumbency. For significant differences see appendix 3, Table 6.

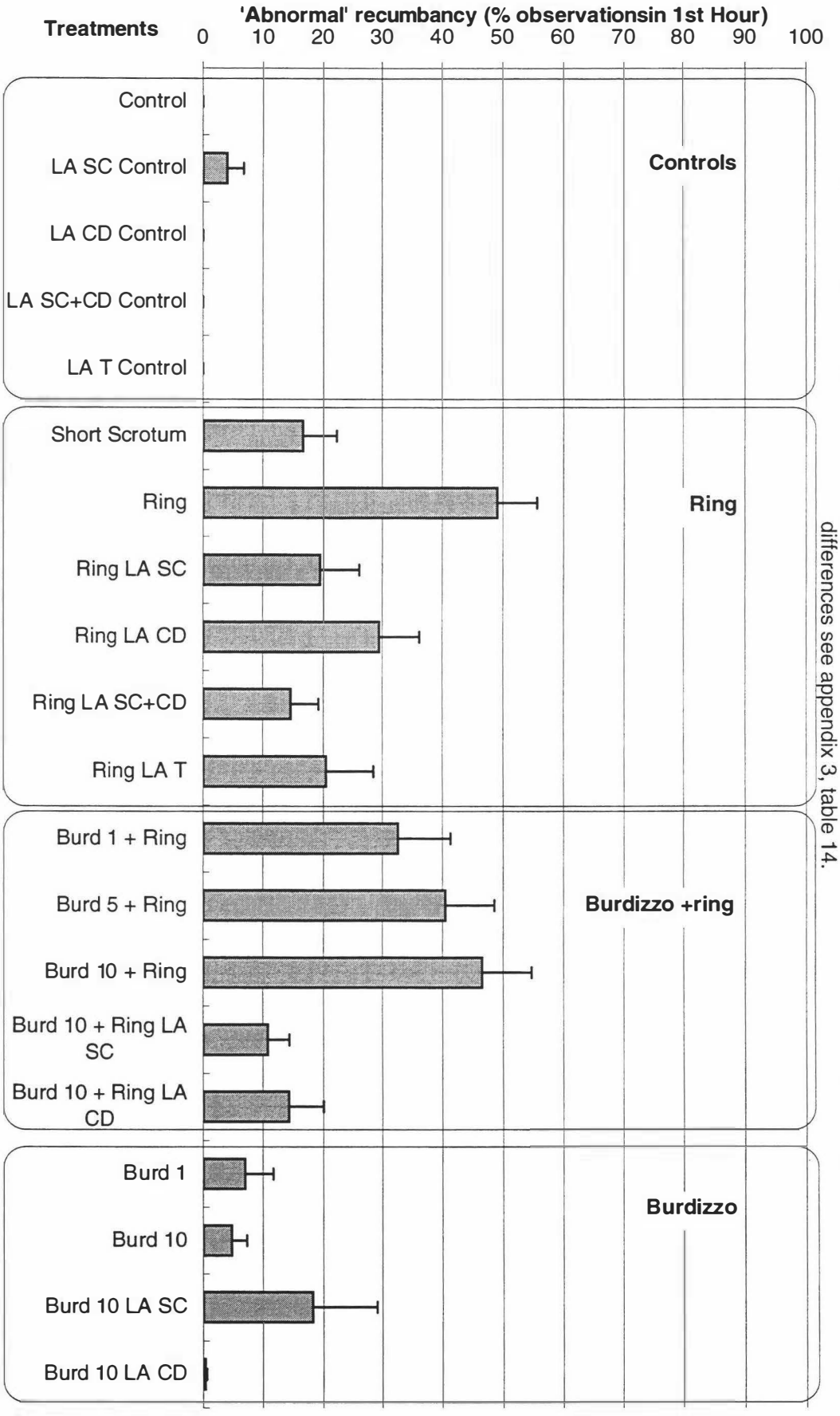


Fig 5.14 The percentage of behaviour (mean ± SEM) in 60 minutes that was abnormal recumbancy. For significant differences see appendix 3, table 14.

Fig 5.7 The percentage of behaviour (mean + SEM) observed in 60 minutes that was abnormal recumbancy. For significant differences see appendix 3, Table 7.

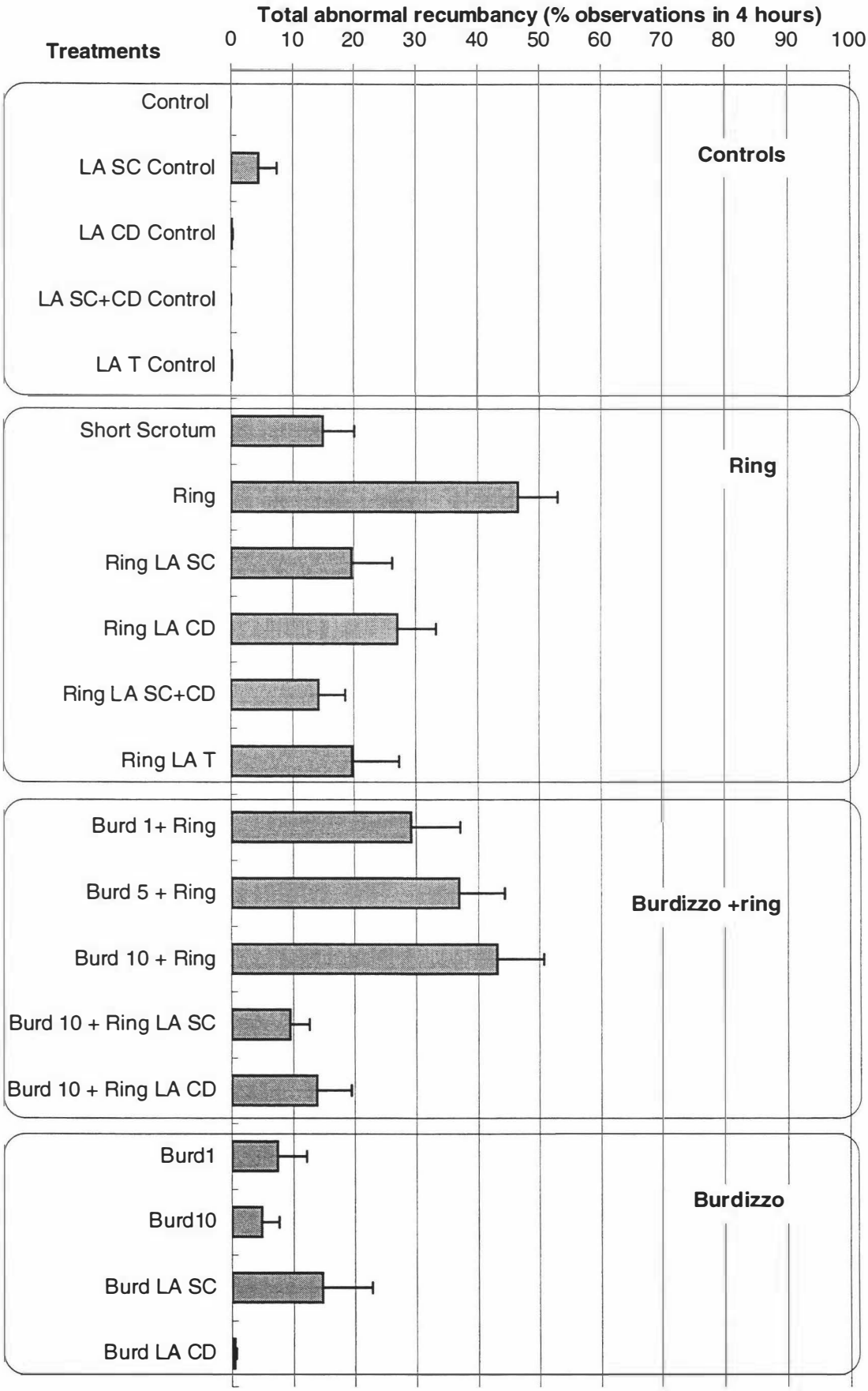


Fig 5.8 The percentage of behaviours (mean + SEM) observed in 4 hours that were abnormal recumbency. For significant differences see appendix 3, Table 8.

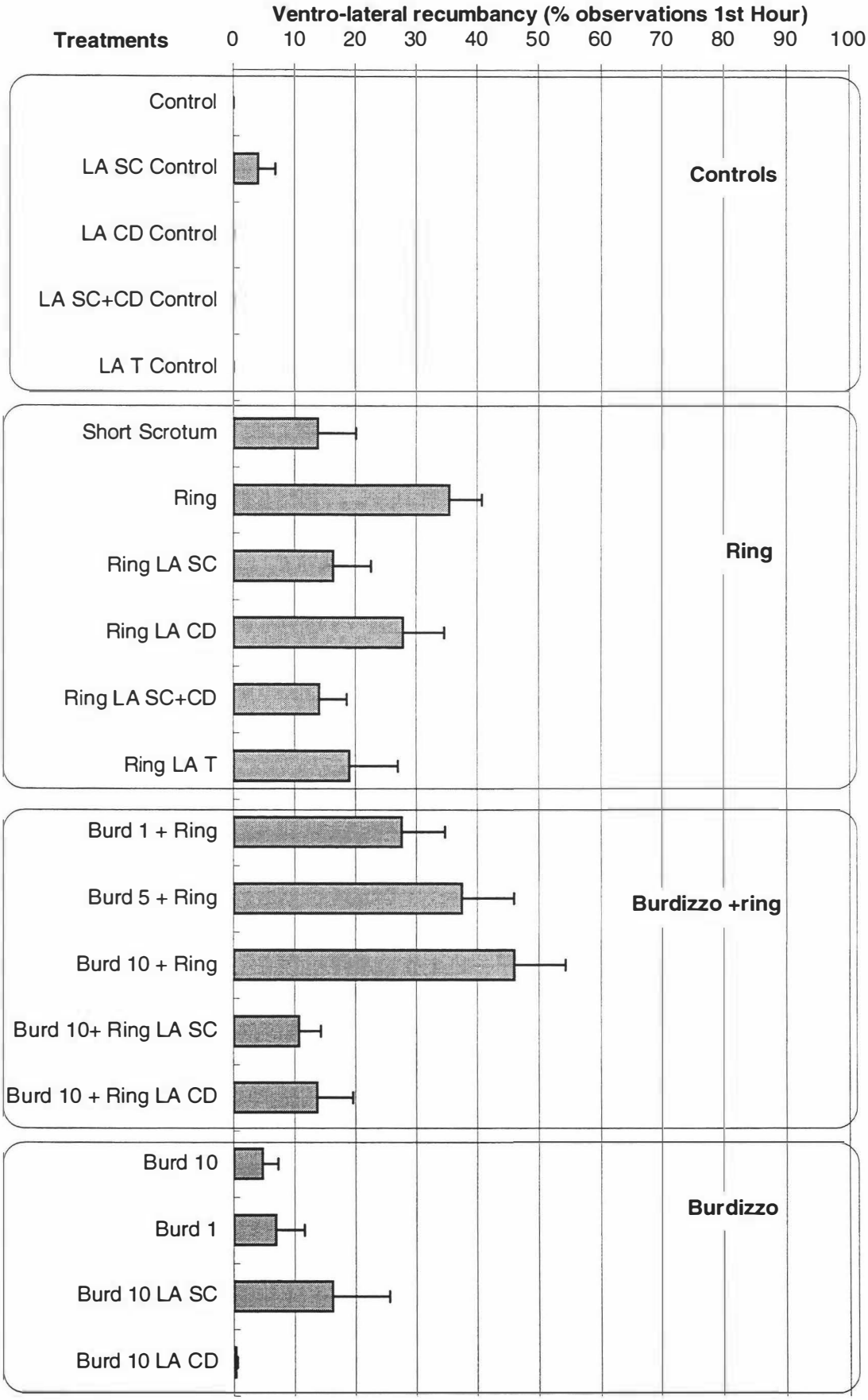


Fig 5.9 The percentage of behaviour (mean + SEM) observed in 60 minutes that was ventro-lateral recumbancy. For significant differences see appendix 3, Table 9.

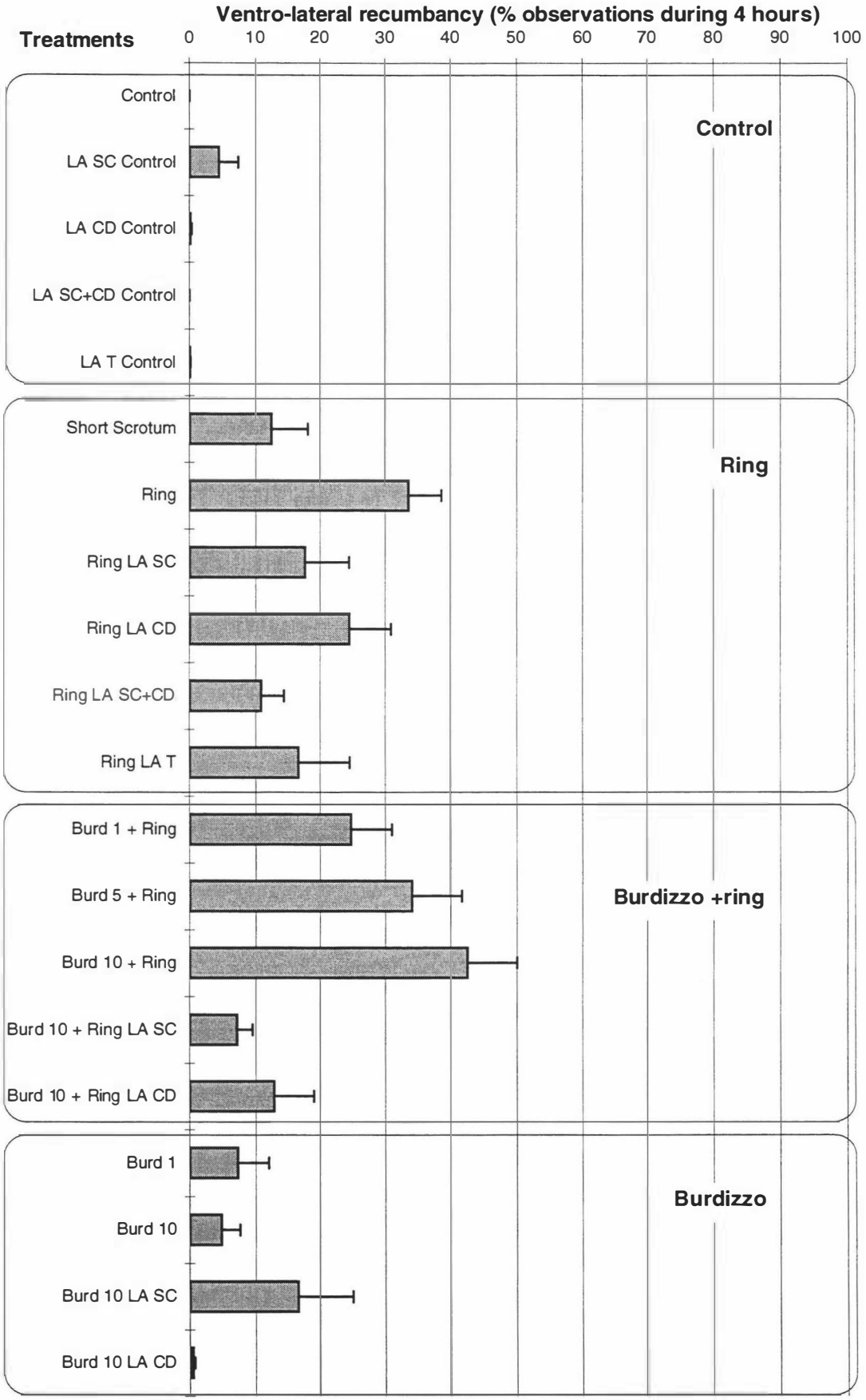


Fig 5.10 The percentage of behaviours (mean + SEM) observed in 4 hours that were ventro-lateral recumbency. For significant differences see appendix 3, Table 10.

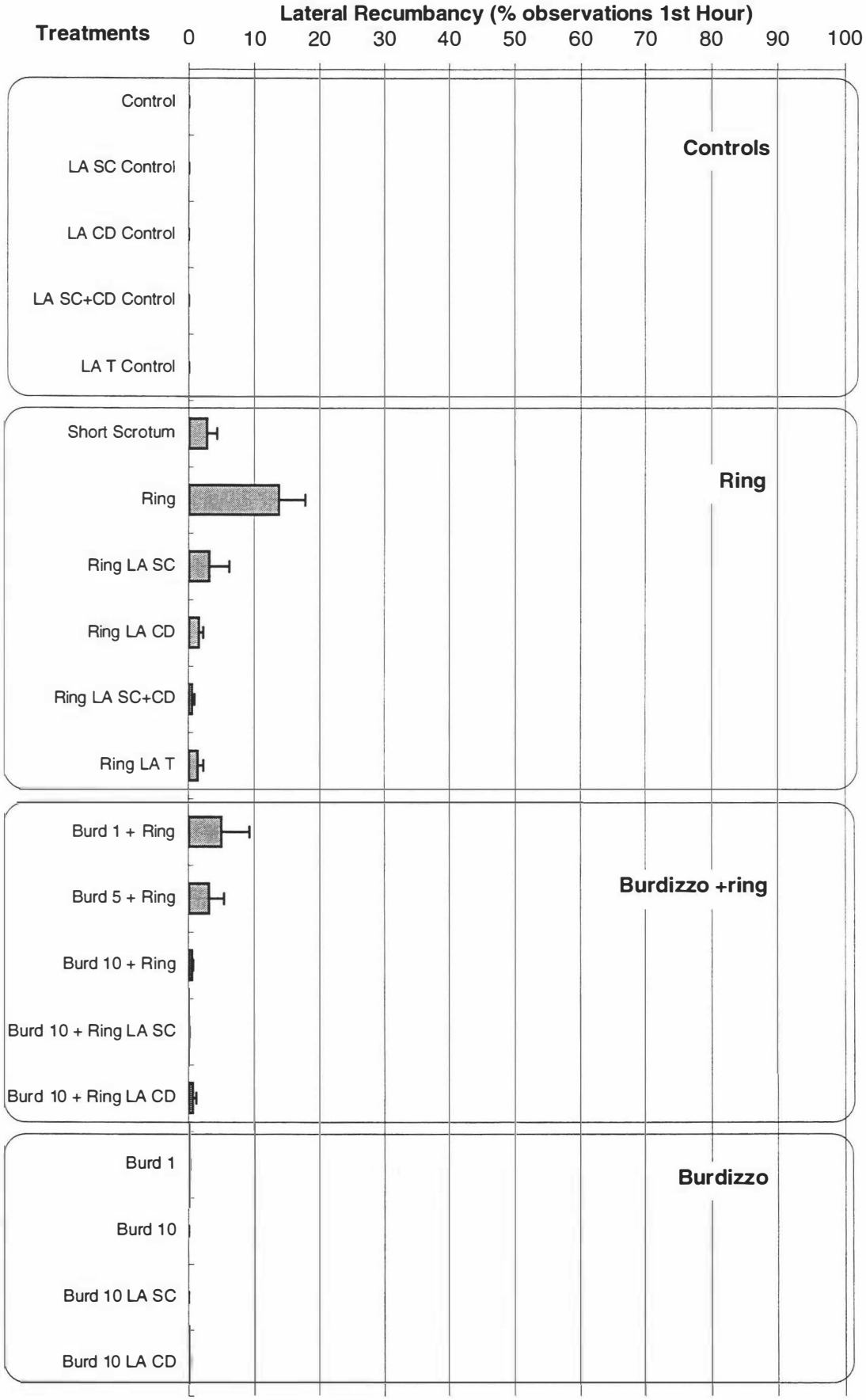


Fig 5.11 The percentage of behaviour (mean + SEM) observed in 60 minutes that was lateral recumbancy. For significant differences see appendix 3, Table 11.

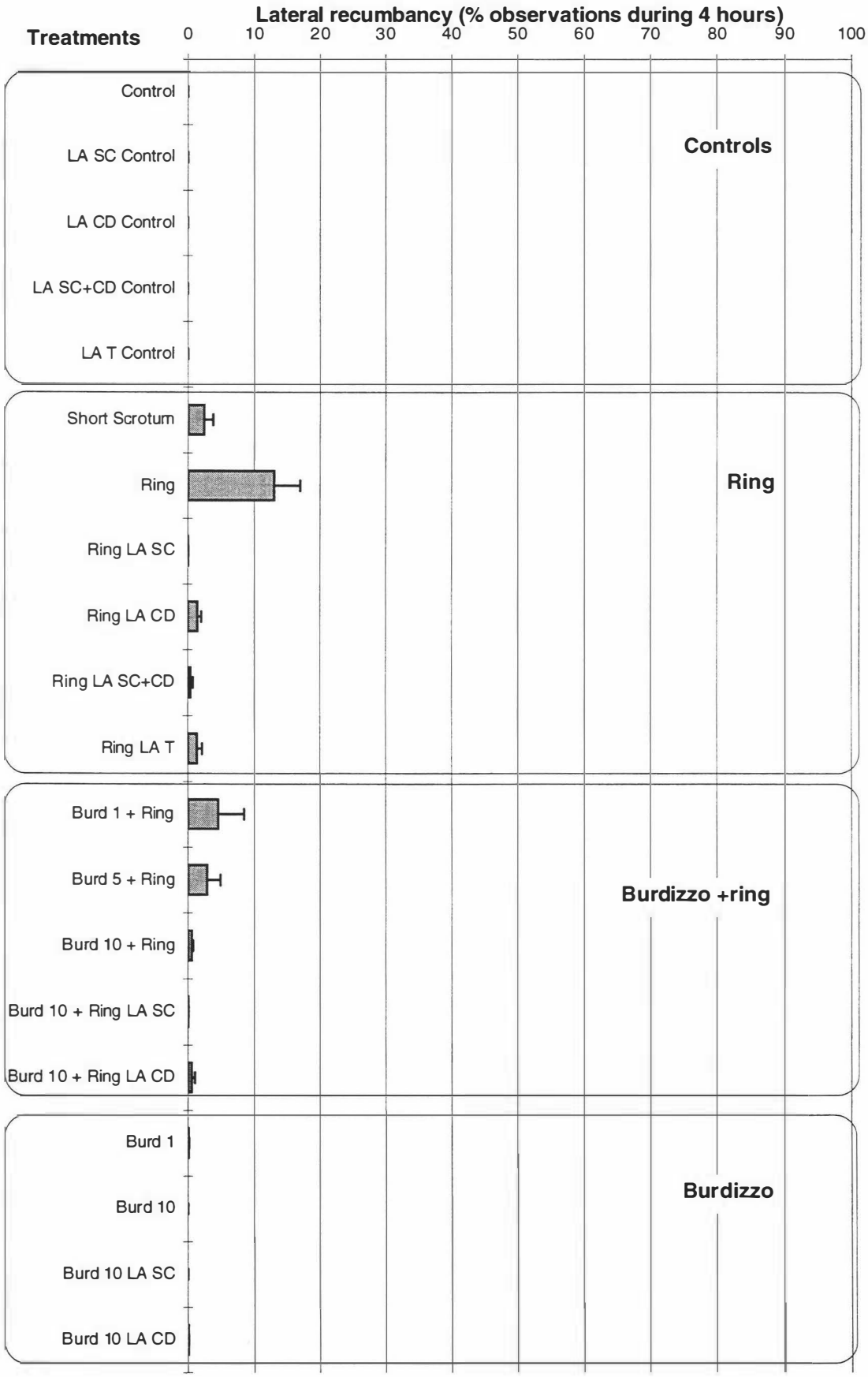


Fig 5.12 The percentage of behaviours (mean + SEM) observed in 4 hours that were lateral recumbency. For significant differences see appendix 3, Table 12.

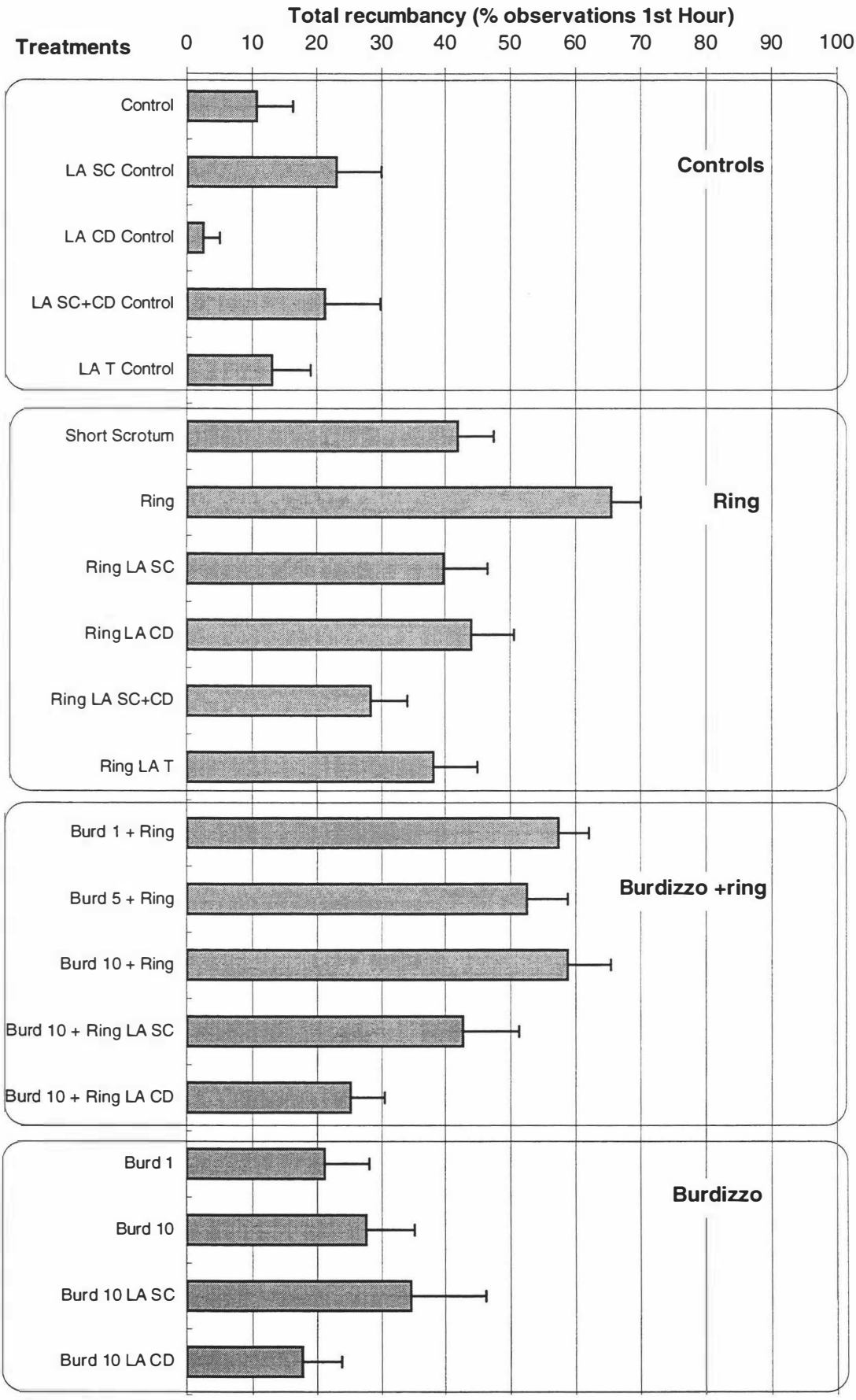


Fig 5.13 The percentage of behaviour (mean + SEM) observed in 60 minutes that was recumbancy behaviour. For significant differences see appendix 3, Table 13.

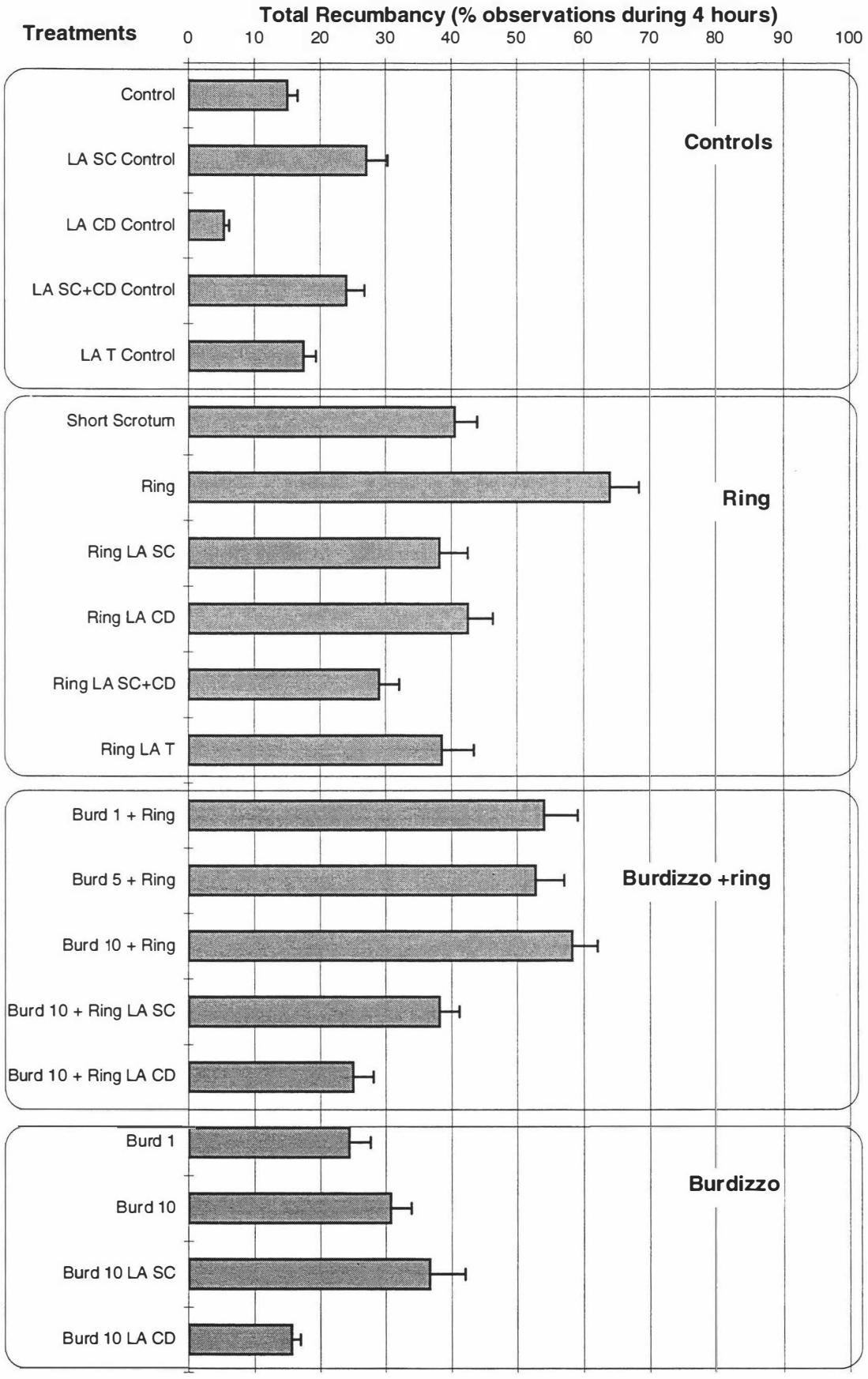


Fig 5.14 The percentage of behaviours (mean + SEM) observed in 4 hours that were recumbency behaviours. For significant differences see appendix 3, Table 14.

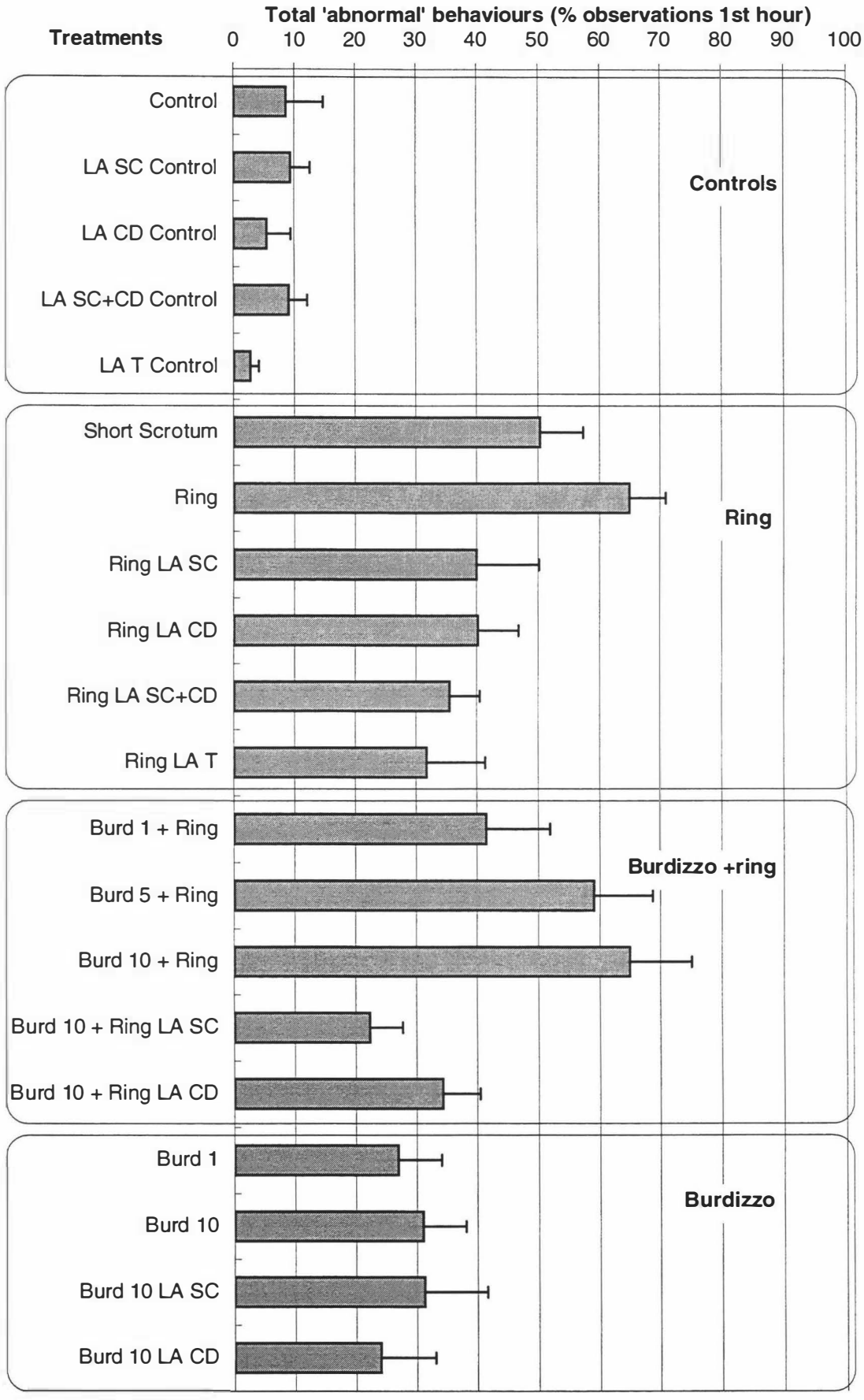


Fig 5.15 The percentage of behaviour (mean + SEM) observed in 60 minutes that was abnormal behaviour. For significant differences see appendix 3, Table 15.

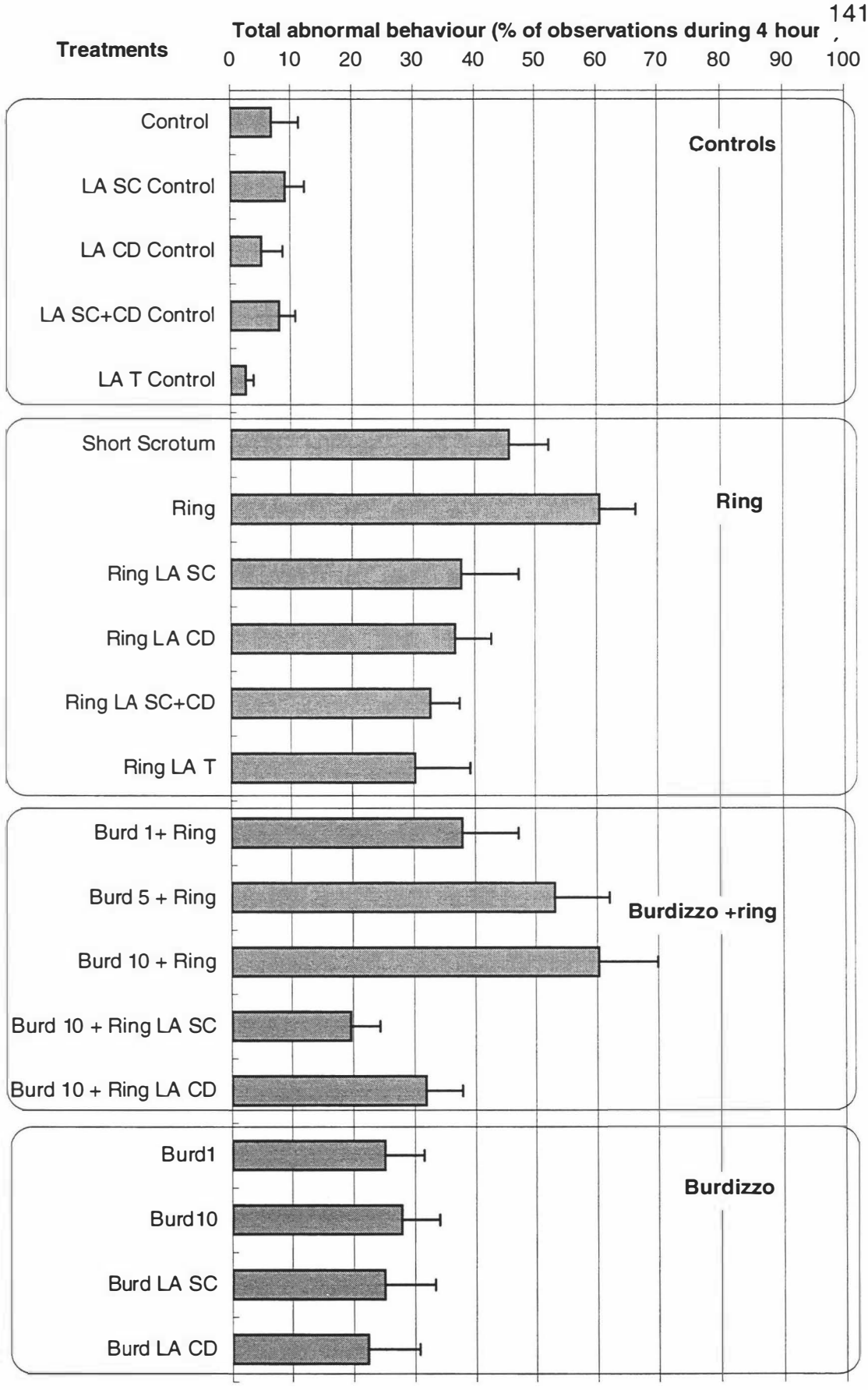


Fig 5.16 The percentage of behaviours (mean + SEM) observed in 4 hours that were recumbency behaviours. For significant differences see appendix 3, Table 16.

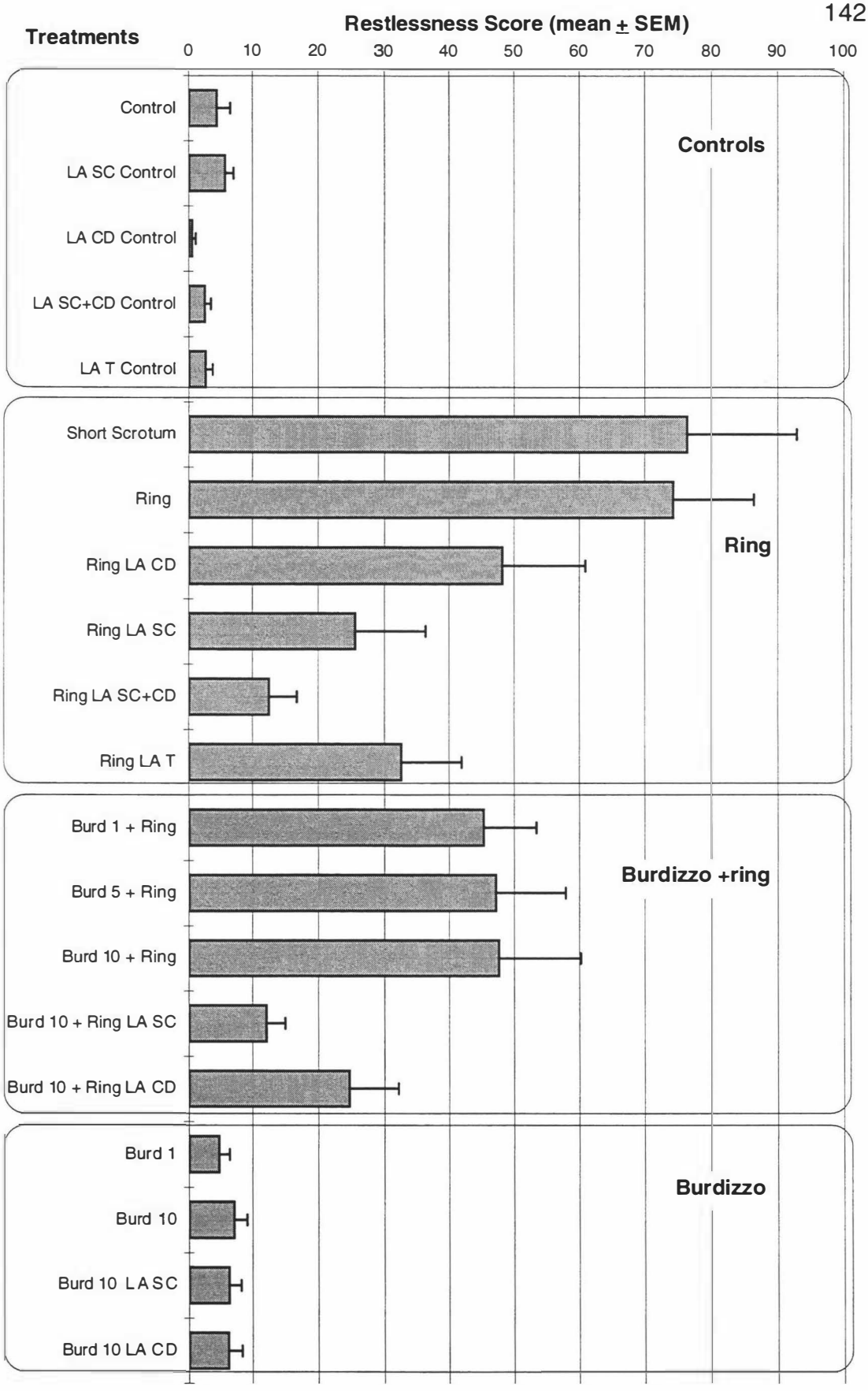


Fig 5.17 The total restlessness score (mean + SEM) in 60 minutes. For significant differences see appendix 3, Table 15.

5.5 DISCUSSION

In the following discussion the usefulness of behavioural parameters to quantify and compare the intensity and duration of pain and/or distress resulting from castration using different methods will be assessed.

It is assumed that, for each method of castration, the behavioural parameters observed can be validated as measures of sensory input, which is presumed to be noxious and result in perception of pain, if the nerve blockade achieved with local anaesthetic returned the behavioural parameters to levels that were similar to those exhibited by control animals.

If the use of local anaesthetic did not return a behavioural parameter to control levels then three explanations are possible.

1. Local anaesthetic did not completely block sensory input from the area affected by the castration. This may be a result of local anaesthetic not reaching all tissues that are sources of sensory input after castration. It also may be a result of some nerves being unaffected by local anaesthetic. It has been suggested that nociception from ischaemic tissues may be able to be transmitted along thick myelinated fibres which may not always be blocked by local anaesthetic (Chabel et al. 1990). The design of the present study did not allow the these alternatives to be distinguished.

2. Lack of sensory input may have been a novel experience and might have elicited the same behaviour as would noxious sensory input from the same area. This may indicate that the particular behavioural parameters are not sensitive to pain sensations only.

   The use of control animals will help to determine the effects of experiencing lack of sensations caused by local anaesthetic. In the present study, lambs that had local anaesthetic injected into the scrotum, spermatic cords, scrotum plus spermatic cords, or the testes and were then handled in the same way as Control lambs, exhibited similar behaviour to the Control lambs. It may therefore be concluded that novel experiences resulting from

local anaesthesia of the areas infiltrated, did not influence the behaviour as measured here.

3. The behavioural parameter was not influenced by sensory input from the anaesthetised area. A behaviour exhibited by an animal after castration may not be a direct result of the sensory input from the area affected directly by castration but some other stimulus caused by the procedure. For example, this may be secondary hyperalgesia of adjacent areas not anaesthetised.

Ring Castration

Ring lambs displayed high levels of 'abnormal' behaviour in the first hour after treatment which was reflected by low amounts of normal standing/walking. This included lateral and ventro-lateral recumbency as well as abnormal standing/walking behaviours. The same lambs were extremely restless during this period. Similar observations have been reported by Mellor and Murray (1989a), Molony et al. (1993) and Lester et al. (1996). The amount of abnormal behaviours and the high restlessness of these lambs may indicate that a significant amount of pain was experienced. By assessing the behaviours of lambs injected with local anaesthetic prior to ring application, it may be possible to determine if the behaviours observed after ring application were a result of sensory input which may be noxious.

Cortisol responses are also helpful in determining the noxiousness of such sensory input and therefore the value of such assessments of behaviour. The cortisol responses of lambs castrated with a ring who had prior injection of local anaesthetic into the scrotum, scrotum plus spermatic cords, or testes, were similar to Control lambs. Because of this, the assumption can be made that Ring LA SC, Ring LA SC+CD, and Ring LA T lambs experienced no noxious sensory input after castration. Although Ring LA CD lambs had a numerically (but insignificantly) reduced cortisol response compared to Ring lambs, this was still significantly greater than the cortisol response of Control lambs. This

suggests that Ring LA CD lambs experienced some form of noxious input after castration.

It is noteworthy that all behaviours returned to normal within four hours after ring castration with or without prior administration of local anaesthetic.

The use of local anaesthetic prior to ring castration resulted in the following effects on behavioural responses:

Completely effective (behaviour similar to Controls)

- A virtual abolition of lateral recumbency in all local anaesthetic + ring treatments.
- Restlessness was reduced to levels similar to controls by using local anaesthetic in the scrotum, or scrotum + spermatic cords.

Partially effective

- A significant reduction in ventro-lateral recumbency in Ring LA SC and Ring LA SC + CD lambs but not Ring LA T or Ring LA CD lambs. However ventro-lateral recumbency was not reduced to control levels in any Ring + LA group.
- A significant increase in the amount of normal standing/walking in Ring + LA lambs. As normal standing/walking was the predominant behaviour exhibited by control animals, any increase in abnormal behaviours was reflected as a decrease in this behaviour. Local anaesthetic given prior to ring application increased the incidences of normal standing/walking but not to control levels.
- A significant reduction in restlessness in all Ring + LA lambs, however Ring LA SC and Ring LA SC+CD lambs were less restless than Ring LA CD or

Ring LA T lambs. However, restlessness was not returned to control values in Ring LA CD or Ring LA T lambs.

Not effective

- There was no significant change in the incidences of abnormal standing/walking behaviours. This may indicate that abnormal standing/walking was not caused by pain resulting from ring castration. The fact that Control lambs exhibited similar amounts of these behaviours also indicates that measurement may be too insensitive to be of value in this context.

Reasons for complete effectiveness

Where local anaesthetic did return a behaviour to control levels, ie lateral recumbency or restlessness (Ring LA SC + CD and Ring LA SC lambs), it may be assumed that the behaviour was indeed only related to sensory input which was successfully removed by the local anaesthetic. However, cortisol responses indicated that Ring LA T as well as Ring LA SC and Ring LA SC + CD lambs probably did not experience noxious sensory input. Because it has been shown that local anaesthetic can remove restlessness (Ring LA SC + CD lambs and Ring LA SC lambs) this would indicate that the restlessness exhibited by Ring LA T lambs is a result of sensory input from the area. However, as there was no cortisol response it seems likely that the sensory input that did result in restlessness in Ring LA T lambs was either not noxious or insufficiently noxious to elicit a cortisol response. This raises the question of whether all restlessness in Ring lambs is actually due to noxious sensory input. Ring LA CD lambs displayed a cortisol response that although reduced in intensity from that in Ring lambs, was not reduced to Control levels. This suggests that the residual restlessness in Ring LA CD lambs could be a result of noxious sensory input (possibly from the scrotum) that is not being blocked by local anaesthetic.

Reasons for partial effectiveness.

There may be more than one explanation as to why local anaesthetic did not completely return normal standing/walking or ventro-lateral recumbency to Control levels. Assuming that local anaesthetic did remove the noxious sensory input (suggested by cortisol responses) then these may be possible reasons.

1. Ventro-lateral recumbency may be not only a result of high level noxious sensory input. Although it is likely that most sensory input is blocked by using local anaesthesia, some tissues may not have been fully anaesthetised and hence some sensation from those tissues may have continued. Although this may not have been sufficiently noxious to cause a cortisol response, it may still have led to some ventro-lateral recumbency. If so, this suggests that ventro-lateral recumbency is a more sensitive index of low level noxious input than cortisol responses. On the other hand, if noxious input is abolished completely by the local anaesthetic this behaviour can apparently be elicited by other stimuli and would be less reliable as an index of pain-induced distress after castration.

   If residual noxious stimulation does remain after local anaesthetic administration which abolished the cortisol response to ring castration (as suggested by the behaviour of Ring LA SC or Ring LA T lambs), this raises the question of how acceptable in welfare terms that presumably low level of noxious input is.

2. Something other than sensory input from the immediate area anaesthetised may also be responsible for the behaviour. It is not possible to determine this from the results of this study.

In the case of restlessness, it was shown that local anaesthetic could reduce high restlessness to control levels, depending on where it was injected. The residual restlessness in Ring LA CD lambs is most likely to have been due to noxious sensory input, a view supported by the cortisol responses of the same lambs which suggested pain-induced distress was being experienced. The most likely source of this noxious input was the scrotum (Chapter 3).

Useful behavioural measures of pain and/or distress after ring castration.

The most useful measures of pain and/or distress after ring castration seem to be restlessness and lateral recumbency. However, lateral recumbency seems to only appear when there is no anaesthetic at all. Any reduction of pain and/or distress using local anaesthetic abolishes this behaviour hence it may only be useful in extreme cases.

Restlessness may be a good measure of sensory input after ring castration. It is also likely (although not conclusive) that it is noxious sensory input that causes high restlessness. It is also important to note that restlessness is only really present in the first hour after treatment and peaks at 30 minutes after treatment (Appendix 3, Fig. 4) compared to the cortisol response of Ring lambs that peaks after 60 minutes. Therefore restlessness may be a good indicator of the onset of pain-induced distress.

Burdizzo + Ring Castration

Burdizzo plus ring lambs exhibited large amounts of abnormal recumbency which was predominantly ventro-lateral during the four hours after treatment. This was reflected in a small amount of normal standing/walking. Lambs exhibited high levels of restlessness after this treatment. To determine if the changes in behaviour after burdizzo and ring application were related to sensory input, prior application of local anaesthetic can be used. Prior local anaesthetic injection into the spermatic cords or scrotum (testes or spermatic cords plus scrotum were not investigated) had the following effects on behaviour parameters:

Completely effective

- The incidences of normal standing/walking were significantly increased by using local anaesthetic. They were increased to levels not significantly

different from controls. This may suggest that normal standing/walking was reduced in burdizzo plus ring lambs because of sensory input caused by burdizzo and ring castration.

- Restlessness was reduced to levels similar to controls by local anaesthetic injected into the scrotum.

Partially effective.

- The amount of ventro-lateral recumbency was reduced but not returned to control levels.

- Restlessness was reduced significantly by local anaesthetic injected into the spermatic cords, however this was not to Control levels.

Not effective.

- No significant effect on abnormal standing/walking was recorded. This may reflect the insensitivity of this measurement.

Reasons for complete effectiveness.

In the case of normal standing/walking lower incidences of this behaviour usually indicate greater incidences of one or more abnormal behaviours such as lateral or ventro-lateral recumbency. When incidences of normal standing/walking are similar to Controls, it suggests that the presence of abnormal behaviours are insignificant. In the case of Burd 10 + Ring LA SC and Burd 10 + Ring LA CD lambs there were still significant amounts of abnormal behaviours such as ventro-lateral recumbency. This indicates the insensitivity of these behaviour measurements caused by variation between animals within each group.

Restlessness was virtually abolished using local anaesthetic injected into the scrotum suggesting that restlessness was caused by sensory input from the

area affected by burdizzo plus ring castration. The cortisol response to burdizzo plus ring castration was also virtually abolished using scrotal local anaesthetic. This suggests that restlessness is indicative of noxious sensory input. There remains the possibility that local anaesthetic abolishes some form of non-noxious sensory input which causes restlessness, but this seems unlikely.

Reasons for partial effectiveness

Restlessness and cortisol responses were not reduced to control levels by injection of local anaesthetic into the spermatic cords prior to burdizzo plus ring castration indicating that the local anaesthetic did not block all noxious sensory input from the area. It is likely that this noxious input was from the ischaemic tissues of the scrotum that were not effected by local anaesthetic. These areas are likely to be innervated by sensory afferent nerve fibres, some of which will pass through the undamaged tissue between the two burdizzo 'cuts'.

It is worth considering whether different behaviours might indicate different types of sensory input (noxious or otherwise) after castration. As ventro-lateral recumbency was only partially reduced by local anaesthetic, it is possible that local anaesthetic injected into the scrotum or spermatic cords did not completely block sensory input from the area. However, that contrasts with the observation that restlessness scores were returned to Control levels by local anaesthetic, thereby suggesting that local anaesthetic did completely block sensory input. It may be that restlessness was triggered by a certain type of sensory input that was removed by using local anaesthetic. On the other hand, ventro-lateral recumbency may have been triggered by other sorts of sensory input transmitted by different types of nerve fibres such as large myelinated nerve fibres which may not be blocked by local anaesthetic (Chabel *et al.* 1990). The results of the present study cannot be used to address this question comprehensively.

Behaviours useful in assessing the intensity of pain and/or distress caused by burdizzo plus ring castration.

Of the behaviours measured in this study, restlessness is probably the best to use to assess the levels of sensory input caused by burdizzo plus ring castration. Although it is very likely, it is not certain that these inputs were noxious. Reductions in normal standing/walking may also indicate sensory input caused by castration with a burdizzo and ring.

Burdizzo Castration

The behavioural response to burdizzo castration could only be characterised by abnormal standing/walking in the first 15-30 minutes after treatment, however the total amount observed in one or four hours after treatment was not significantly different to Control values. Very little abnormal recumbency was observed and the incidences were similar to Control values. Normal standing/walking was significantly lower than in Controls. This was the only behavioural parameter measured that was different to Controls and therefore the only behaviour that could be influenced by local anaesthetic. Restlessness was not a feature of Burdizzo lambs.

Local anaesthetic had no significant effect on any behavioural parameter that was significantly different from Control values.

Reasons for no effect of local anaesthetic on behavioural parameters.

- Local anaesthetic was not effective in reducing sensory input caused by burdizzo castration. Because local anaesthetic injected into the scrotum or spermatic cords had no effect on any behavioural parameter it may be suggested that local anaesthetic did not reduce the amount of sensory input after burdizzo castration. As local anaesthetic did not affect the cortisol responses to burdizzo castration this might be the case. However, local anaesthetic has been shown to reduce the cortisol response to burdizzo castration in another study (Molony 1993), which suggests that at least some, if not all of the cortisol response was due to sensory input from damaged tissues.

- No change in sensory input was elicited by burdizzo castration. If burdizzo castration did not cause any change in sensory input from the damaged

tissues, then local anaesthetic would not be expected to alter the behavioural response. However, intuitively this does not seem likely. Furthermore, the fact that local anaesthetic has been shown to reduce the cortisol response to burdizzo castration in one study (Molony, 1993), although not here, casts doubt on this theory. The cortisol response to burdizzo castration (Chapter 3) indicated that either there was a significant amount of noxious sensory input that lasted for a period of almost four hours. However, it may be that the cortisol response was caused by something other than sensory input. For reasons discussed in Chapter 3 sensory independent stimulation of the hypothalamic-pituitary-adrenal axis does not seem likely but cannot be ruled out.

- Burdizzo castration may cause subtle changes in behaviour that were not detected by the observers in this study, hence any effects of local anaesthetic would not be noticed. Burdizzo castration has been shown to result in statue standing (Kent *et al.* 1995) which can be hard to differentiate from normal standing. Abnormal standing/walking was observed in this study but the amounts were not significantly different from control values. In the present study no statue standing was recorded in burdizzo lambs, which suggests that observers in this study had difficulty in differentiating normal standing from statue standing. The difficulty in differentiating between statue standing and normal standing does however demonstrate that some behaviours are very hard to identify and that there may be behaviours exhibited by animals after treatments that are so subtle that they are not detected by observers.

Useful behaviours when assessing the pain and/or distress caused by burdizzo castration.

Although incidences of normal walking and standing exhibited by Burdizzo lambs were significantly different from Control values, the fact that they were not affected by local anaesthetic may suggest that this parameter cannot be used reliably. A behavioural parameter that clearly indicates that pain and/or

distress are being experienced by burdizzo castrated lambs has therefore not been identified in this study.

Comparisons of relative levels of pain and/or distress caused by castration using different methods.

The results of this and other behaviour studies lead to the following conclusions about conditions which need to be met in order to use behaviour to compare the pain and/or distress caused by different treatments.

1. It must be validated that a behavioural change is indicative of pain and/or distress caused by a treatment. This can be achieved using controls and local anaesthetic studies as described above.

2. Treatments can only be compared if they elicit responses with behaviours in common. Because it is not known whether particular behaviours indicate more pain than others, treatments that elicit unique behaviours cannot be a basis for meaningful comparison. It is likely that the sensations experienced by animals after different treatments will not be the same if the treatments damage different tissues (castration cf tailing) or the same tissues are damaged in a different way (cutting cf ischaemia). Different experiences have been shown to result in vastly different behavioural responses such as surgical castration which caused immobility, compared with ring castration which caused high restlessness (Lester et al. 1996).

3. Treatments may be compared if they are similar in form. If a treatment is modified in some way it would be expected that at least some of the tissue damage would be similar and hence a comparison may be possible. Using this line of reasoning, comparisons may be able to be made between ring castration and burdizzo plus ring castration, as well as burdizzo castration and burdizzo plus ring castration. However, these comparisons can only be made using behavioural responses which have features in common in both groups.

The relative intensities of pain and/or distress caused by ring castration compared to burdizzo plus ring castration.

Using the guidelines described above, this comparison can be made using restlessness. When using restlessness as an index, there was no significant difference between the intensities of pain and/or distress caused by burdizzo plus ring castration and ring castration.

The relative intensities of pain-induced distress caused by ring castration and short scrotum creation.

As short scrotum creation is very similar to ring castration it is possible to compare the behavioural responses to these two treatments. Although local anaesthetic was not used with short scrotum creation the same behaviours that have been determined to be indicative of changes in sensory input caused by ring castration should be able to be used for short-scrotum creation. Short scrotum creation caused as much restlessness as ring castration and hence it may be presumed as much sensory input. However, unlike the responses to ring castration, lateral recumbency was not evident in short scrotum lambs which may indicate that sensory input in these lambs may be less noxious.

The relative intensities of pain and/or distress caused by burdizzo castration and burdizzo plus ring castration.

As there were no similar behavioural parameters identified it does not seem possible to compare the intensities of pain and/or distress caused by burdizzo or burdizzo plus ring castration. Although it may be suggested from the results of this study that burdizzo castration causes little or no pain and/or distress and hence will cause less than the burdizzo plus ring method, this is questionable.

Comparisons that cannot be made.

Using the guidelines determined above, it is not possible to compare burdizzo castration with ring castration meaningfully using behaviour alone because of the differences in damage inflicted and the unique behavioural responses. Another method of analysing pain and/or distress needs be used.

Comparing durations of pain and/or distress caused by treatments.

If a behaviour is identified as being indicative of pain it can be used to determine the duration of the pain caused by a treatment. Although different treatments may cause different types of behaviour and hence the intensity of pain cannot be compared, the period during which each behaviour is present can be.

However in the case of Burdizzo castration, as no behaviour was identified as being indicative of pain, the duration of pain may be hard to determine using the behaviours measured in this study.

Ring castration, short scrotum creation, and burdizzo plus ring castration seemed to have very similar durations of sensory input. Restlessness scores indicate that sensory input caused by these treatments had virtually returned to normal levels after one hour. Other abnormal behaviours, such as ventro-lateral recumbency, although not necessarily caused by noxious or strongly noxious sensory input, disappeared 3 hours after treatment.

CHAPTER 6: GENERAL SUMMARY AND CONCLUSIONS

The major original findings of this study were as follows:

Ring Castration:

1. Both the scrotum and the testes are sources of noxious sensory input (probably nociception) after ring application. This was supported by the following findings.

   A. Short scrotum creation, which does not apparently change sensory input from the testes (Cottrell and Molony, in press) resulted in a cortisol response which was less in magnitude than that caused by ring castration but more than in Controls.

   B. The cortisol response to ring castration with prior injection of local anaesthetic into the spermatic cords was similar to that caused by short scrotum creation. Although the cortisol response in Ring LA CD lambs was numerically lower than in Ring lambs this difference was not significant.

   C. The cortisol response to ring application was abolished using local anaesthetic injected into the scrotum, spermatic cords plus scrotum, or testes.

   D. Local anaesthetic injected into the testes probably leaked out and anaesthetised at least parts of the scrotum. Local anaesthetic injected into the scrotum probably also anaesthetised the surface of the testes.

2. Lateral recumbency and restlessness were found to be the only behaviours to be prevented using local anaesthetic and hence the only behaviours that could be established as being exclusively caused by sensory input from the affected area. Although it was not able to be

conclusively demonstrated that it was *noxious* sensory input that caused high restlessness, intuitively this seems highly likely.

3. Ventro-lateral recumbency was at least partly influenced by changes in sensory input from the area, however it could have been influenced by some other factors. This was not established.

Burdizzo plus ring castration:

1. Burdizzo plus ring castration caused a plasma cortisol response that peaked at 30 minutes and had returned to pre-treatment levels by 150 minutes after treatment. This was a more immediate response than that exhibited by ring castration, however there were no significant differences between their respective peak values and integrated cortisol responses.

2. The duration of burdizzo application (1, 5, or 10 seconds) did not have any significant effects on the durations or magnitudes of the cortisol responses to burdizzo plus ring castration.

3. The burdizzo used in the conventional manner did not reduce the distress (as indicated by plasma cortisol levels) caused by ring castration as was intended. The conventional manner of burdizzo application (one "cut" over each spermatic cord) when used alone to castrate lambs leaves a large portion of scrotal tissue undamaged to prevent atrophy and sloughing of the scrotum. This tissue is likely to remain innervated and hence nociception from ischaemic tissue created by application of a ring is able to be transmitted via afferent nerve fibres that pass through the undamaged tissue.

4. The plasma cortisol response to burdizzo plus ring castration can be abolished by injecting local anaesthetic into the scrotum but only reduced by injecting it into the spermatic cords.

5. The use of the burdizzo on the tail did not alter the cortisol response to burdizzo plus ring castration with ring tailing.

6. Restlessness was the only behaviour to be established as being caused exclusively by changes in sensory input from the area affected by the

treatment. Although it cannot be conclusively demonstrated that the changed sensory input that caused restlessness was noxious, intuitively this seems likely.

Burdizzo castration:

1. Burdizzo castration elicited a cortisol response that peaked at 30 minutes and lasted for 180 minutes after treatment. The magnitude was not significantly different from that exhibited by lambs castrated with a ring.

2. The duration of burdizzo application (1 or 10 seconds) did not have any significant effect on the magnitude or duration of the cortisol response. It also did not alter the behavioural response.

3. Local anaesthetic injected into the spermatic cords or into the scrotum did not have any significant effect on the cortisol response to burdizzo castration. It also had no effect on the behavioural response.

4. The behaviour exhibited by lambs castrated by a burdizzo was not significantly different from that of Control lambs. It is doubtful that this can be interpreted as meaning that no changed sensory input was caused by burdizzo application. It is likely that any behavioural response exhibited by these lambs was too subtle to be detected by the methods of observation used here.

General

Different behaviours were found to indicate changes in sensory input after different treatments. These individual behaviours were useful for comparing similar treatments, such as ring castration or ring castration with prior local anaesthetic injection or burdizzo plus ring castration. However, when treatments caused different tissue damage and different behavioural responses, meaningful comparisons could not be made using behaviour alone.

Practical implications

The conventional use of the burdizzo after ring castration as performed in this study, cannot be recommended as a way of reducing the distress caused by ring castration. It seems that to successfully reduce the pain-induced distress caused by ring castration, the burdizzo must be used in the unconventional manner of one application across the entire width of the scrotum as described by Kent *et al.* (1995). However there may be some complications of this method of burdizzo use that have not been assessed in the present study or in that described by Kent *et al.* (1995) such as tetanus and gangrene. Also the increased time taken to perform the procedure needs to trialed in the field when castrating a large mob of lambs. The effectiveness of different types of burdizzo also need to be assessed to discover the effects of different jaw dimensions and pressures applied. Further to this, laboratory tests, assessing the activity of isolated nerves within the spermatic cord and scrotum after burdizzo application, need to be performed to confirm that the burdizzo is actually preventing nociception. Therefore, more research is needed before the use of the burdizzo in combination with ring castration as described by Kent *et al*. (1995) could be recommended.

To thoroughly assess the modified method of burdizzo application (across the full scrotal width) plus rings, a study involving larger numbers of lambs (at least 20) needs to be performed using a similar protocol to the study described in this thesis. If this study supported the beneficial effects of such burdizzo application with rings, then a larger study would need to be performed under field conditions involving farms from all over the country. This study must be designed to detect and assess any hazards of this method of castration such as gangrene, tetanus and chronic pain and compared with incidences of hazards caused by ring castration and tailing.

Local anaesthetic injected into the scrotum, spermatic cord + scrotum, or testes can apparently reduce the amount of pain or discomfort caused by ring castration. However, before the use of local anaesthetic in field conditions can

be recommended, further research must be performed to assess the risk of sepsis. The injection of local anaesthetic 15 minutes prior to castration is clearly not practical in field conditions. Therefore more work needs to be done to assess the effectiveness of local anaesthetic injected at the same time as castration and tailing are performed. A modified method of local anaesthetic injection into the testes and lower part of the scrotum with ring application may be successful in reducing pain while still being practicable in large scale farm operations, however this is yet to be determined.

Experimental Design and Limitations

A limitation of this study was the inability to assess the full extent of local anaesthetic diffusion after its injection into the scrotum, testes or spermatic cords because of the likely different diffusion properties of lignocaine and the dye mixed with it. However the extent to which the dye diffused (Chapter 2) probably indicates the minimum diffusion capabilities of the local anaesthetic.

A possible criticism of the castration studies described here was that animals were penned for long periods and the lambs and their mothers separated in what would be considered to be unnatural conditions. As pre-treatment plasma cortisol concentrations were usually low and plasma cortisol concentrations returned to levels similar to pre-treatment levels after castration it may be considered that being separated did not influence the cortisol concentrations markedly. Furthermore, no lambs exhibited high pre-treatment cortisol concentrations that would indicate distress caused by separation, except those excluded for that reason (Appendix 1).

Repeated handling may be considered to be an added stressor. Although Lester et al. (1991b) have demonstrated that this was not the case after surgical castration, repeated handling could have caused more prolonged elevations in cortisol concentrations in the less severe procedures examined here. This was not examined experimentally in the present study.

It may be argued that as there were two lambs in each bleeding pen, sampling order may influence cortisol results. It has been demonstrated that in a pen containing several calves sampling order did not significantly alter cortisol results (Petrie 1994). Furthermore, as each lamb usually took only 15 seconds to bleed, people were only usually present in the pen for 1 minute. This is far shorter than the time required for a stressor to elevate the concentration of cortisol in the jugular blood of lambs, as indicated by the present study and all similar previous studies. Hence bleeding order would not be expected to influence the cortisol results.

Another criticism may be that true basal pre-treatment cortisol concentrations may not have been obtained. Although this may be the case, the base-line samples were on the whole acceptably low indicating that animals were minimally stressed. Some lambs that had an extremely high baseline sample were excluded from the study (Appendix 1).

A limitation of the behaviour study was that blood samples were taken from the lambs during the observation period. As the presence of the bleeders in the pen altered the behaviour of the lambs, all behaviour during the period the handlers were in the pen and immediately after were not recorded. The continued disturbance of the lambs may have affected the behavioural results.

A further possible limitation of the behavioural study, may be that some change in behaviour may have been too subtle for our behavioural observation methods to detect. Therefore behaviours reflecting a change in sensory input caused by the treatment may have been scored as normal behaviours, and hence the distress underestimated.

Personal Comments

New Zealand farmers operate in a market driven economy which is largely influenced by international pressures. As the international spotlight is turned onto the animal welfare implications of farming practises in New Zealand, farmers must be prepared to modify and improve these practises to gain

international approval and avoid potential trade barriers. Although a reality, it is sad that it is economic pressure, rather than ethical conviction that apparently is the impetus for improving farm animal welfare. However, this is not to say that farmers are not concerned about the animals under their care.

There are two avenues to explore when assessing and minimising the welfare concerns created by castration of lambs.

First and foremost, farmers and the meat industry must work together to minimise the need to castrate ram lambs by either killing lambs before they reach puberty, or adjusting farm management to accommodate entire males.

Secondly, when castration is unavoidable, practicable methods of minimising the pain caused by castration must be researched and put into practise. These would include the development of efficient methods of using local anaesthesia and analgesia which, once devised, should be required by regulation. Achieving this will involve cooperation between researchers, the Ministry of Agriculture, farmers and the meat and pharmaceutical industries.

APPENDIX 1: PLASMA CORTISOL CONCENTRATIONS (NMOL/L) IN LAMBS AFTER DIFFERENT TREATMENTS (CHAPTER 3).

APPENDIX 1(A): CORTISOL CONCENTRATIONS OF LAMBS EXCLUDED FROM CHAPTER 3.

		Time											
Treatment	Lamb	-15	0	15	30	45	60	90	120	150	180	210	240
Control	L23	15	68	20	15	13	26	16	7.5	26	11	13	19
LA SC + CD Control	L69	1	10	17	70	90	52	42	80	34	61	63	99
Short Scrotum	L89	1	1	1	69	128	174	162	180	61	27	19	16
Ring LA SC	L152	1	9	22	9	20	29	18	60	113	76	63	75
Ring LA CD	L171	80	46	104	12	74	53	40	15	6	1	2	3
Ring LA T	L15	9	19	36	39	54	60	95	102	46	46	46	9
Burd 10 LA CD	L70	35	33	65	123	232	180	306	167	253	325	224	141

Table A1.1 The plasma cortisol concentrations (nmol/l) of the lambs excluded from different treatment groups. The lambs were excluded on the basis that the cortisol concentrations were at least 2 standard deviations different from the mean value of the cortisol at three different sampling times.

APPENDIX 1(B): PLASMA CORTISOL CONCENTRATIONS (NMOL/L) IN LAMBS (CHAPTER 3).

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
ACTH	L105	17	30	72	75	82	92	90	67	31	26	24	19
ACTH	L123	3	3	58	58	73	89	90	101	82	58	34	16
ACTH	L144	20	29	116	114	125	132	114	65	30	13	12	11
ACTH	L149	0	1	65	103	89	103	102	109	94	73	34	19
ACTH	L177	8	9	60	75	103	108	159	75	43	15	15	21
ACTH	L26	16	28	102	82	87	80	130	96	107	82	79	51
ACTH	L57	0	9	46	90	85	64	67	65	30	21	19	16
ACTH	L7	8	2	93	168	169	151	145	139	81	29	29	35
ACTH	L90	11	8	99	123	137	160	153	68	41	26	39	20
Control	L141	2	14	15	10	5	13	15	8	15	6	9	8
Control	L150	9	31	46	28	35	44	46	29	51	29	9	13
Control	L16	10	57	27	22	12	15	9	9	12	6	6	6
Control	L184	1	23	1	1	0	0	0	1	1	0	3	1
Control	L2	17	42	32	51	14	17	19	20	20	20	30	17
Control	L59	7	7	2	3	2	11	5	10	6	1	17	1
Control	L71	3	19	30	20	5	4	14	4	4	5	3	6
Control	L96	13	17	45	16	5	14	2	7	3	2	1	2

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
LA SC Control	L128	26	35	43	29	27	14	17	38	30	26	17	23
LA SC Control	L153	73	67	70	73	95	65	74	55	89	49	70	104
LA SC Control	L162	7	3	4	5	4	14	5	6	6	3	22	7
LA SC Control	L178	15	13	9	5	5	8	6	9	2	1	5	3
LA SC Control	L38	2	16	6	3	1	5	6	9	8	8	12	10
LA SC Control	L41	8	15	16	9	15	16	12	10	13	14	13	15
LA SC Control	L63	26	9	14	10	16	16	28	23	16	11	15	14
LA SC Control	L87	0	26	7	5	8	6	2	5	2	32	2	1
LA SC Control	L98	32	65	79	42	22	43	32	14	19	13	13	1
LA CD Control	L134	5	34	36	21	6	21	9	3	5	4	8	2
LA CD Control	L14	28	39	28	5	9	17	16	6	6	12	11	3
LA CD Control	L155	35	107	90	0	3	5	10	0	0	2	2	9
LA CD Control	L165	13	39	52	23	12	8	27	31	19	19	14	8
LA CD Control	L33	3	29	14	5	1	5	8	1	6	5	1	1
LA CD Control	L52	8	23	41	14	8	6	16	6	7	12	5	12
LA CD Control	L54	6	13	19	15	6	21	8	10	10	13	4	14
LA CD Control	L82	12	14	23	14	9	8	3	49	30	1	2	1
LA CD Control	L94	0	16	12	2	2	3	2	0	6	0	2	1

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
LA SC + CD Control	L108	9	8	27	24	20	31	10	0	3	9	8	1
LA SC + CD Control	L13	15	14	20	15	43	46	32	39	35	20	20	12
LA SC + CD Control	L132	2	15	17	8	10	14	15	9	8	6	8	6
LA SC + CD Control	L147	0	15	16	9	5	12	19	4	19	9	15	5
LA SC + CD Control	L181	3	12	1	1	1	1	0	0	10	0	0	2
LA SC + CD Control	L4	22	43	36	39	35	34	34	33	38	35	36	46
LA SC + CD Control	L53	7	36	55	32	22	17	32	12	13	21	23	7
LA SC + CD Control	L79	3	3	8	6	5	2	6	3	6	4	1	3
LA T Control	L114	0	2	1	1	2	1	0	0	0	1	1	5
LA T Control	L117	16	16	10	6	6	16	27	12	15	7	7	12
LA T Control	L131	13	10	5	3	14	19	6	11	7	2	20	4
LA T Control	L139	4	12	2	1	1	11	8	1	12	3	16	12
LA T Control	L145	28	41	32	21	22	30	21	21	12	19	13	11
LA T Control	L186	11	8	7	10	10	9	7	4	4	8	6	15
LA T Control	L34	5	21	17	3	6	19	20	3	0	1	0	1
LA T Control	L6	2	5	9	10	21	16	9	14	11	5	12	17
LA T Control	L88	2	2	4	3	2	5	4	2	4	0	2	1

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
Short Scrotum	L104	19	20	14	12	42	73	84	48	7	7	23	19
Short Scrotum	L116	10	27	28	23	22	35	14	12	19	10	3	4
Short Scrotum	L161	2	21	43	29	15	24	82	17	9	6	1	15
Short Scrotum	L180	0	2	13	26	24	42	60	58	16	14	5	2
Short Scrotum	L25	8	72	95	45	52	102	53	42	30	46	65	17
Short Scrotum	L3	5	20	32	37	59	58	29	16	16	12	20	29
Short Scrotum	L44	11	6	7	5	19	29	30	12	7	8	5	0
Short Scrotum	L47	7	10	23	17	28	46	56	37	15	3	5	15
Ring	L137	11	11	11	20	56	64	30	29	27	3	23	14
Ring	L148	0	3	0	50	57	74	43	31	26	1	3	6
Ring	L166	13	17	34	49	61	86	66	55	21	20	6	1
Ring	L30	2	0	12	46	49	53	55	24	28	24	16	5
Ring	L55	4	10	21	28	44	63	61	22	6	7	3	3
Ring	L9	3	12	36	61	81	87	81	81	52	15	10	10
Ring	L91	3	0	1	24	43	78	45	55	6	3	2	43
Ring	L95	0	2	10	12	46	68	93	36	14	1	0	0
Ring	L99	0	0	5	3	21	55	63	24	23	9	0	0

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
Ring LA SC	L113	5	4	3	5	7	12	4	11	4	1	0	1
Ring LA SC	L12	6	5	17	26	26	28	31	23	15	16	17	10
Ring LA SC	L135	3	8	17	30	46	56	35	19	12	6	6	0
Ring LA SC	L175	8	18	12	10	26	13	17	14	13	12	17	16
Ring LA SC	L37	8	23	14	12	38	16	8	14	24	17	8	29
Ring LA SC	L51	6	5	10	5	11	13	8	6	5	5	3	5
Ring LA SC	L73	7	14	36	31	50	31	7	8	17	6	3	13
Ring LA SC	L93	2	1	0	7	9	2	14	3	2	1	5	23
Ring LA CD	L107	2	5	31	24	78	103	97	28	10	38	5	1
Ring LA CD	L143	1	6	21	19	8	3	4	2	1	3	1	1
Ring LA CD	L160	2	2	12	19	10	44	24	3	16	1	0	0
Ring LA CD	L20	13	10	28	35	44	46	41	22	13	13	10	10
Ring LA CD	L24	8	9	41	45	21	32	59	93	61	6	78	5
Ring LA CD	L64	3	7	5	7	5	9	13	9	8	6	3	5
Ring LA CD	L67	11	19	10	13	52	68	68	25	16	16	5	5
Ring LA CD	L85	3	3	3	3	44	66	27	22	19	2	51	2

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
Ring LA SC+CD	L115	3	3	0	1	6	8	0	8	3	0	3	0
Ring LA SC+CD	L133	1	10	14	10	9	19	5	2	8	5	6	1
Ring LA SC+CD	L156	0	16	10	7	4	2	1	0	0	0	2	0
Ring LA SC+CD	L158	0	1	6	1	0	4	9	6	0	1	1	1
Ring LA SC+CD	L187	15	26	27	17	22	13	10	10	10	7	11	9
Ring LA SC+CD	L21	16	23	17	19	72	58	35	20	15	15	11	12
Ring LA SC+CD	L35	1	1	2	16	24	43	9	7	7	1	5	1
Ring LA SC+CD	L72	9	7	15	15	35	30	13	40	32	17	21	13
Ring LA SC+CD	L97	8	39	38	19	11	26	35	7	21	9	0	15
Ring LA T	L100	0	5	14	24	23	35	23	12	5	1	5	0
Ring LA T	L168	6	30	23	9	42	31	9	17	16	5	24	5
Ring LA T	L183	19	24	9	0	5	8	1	1	0	0	5	0
Ring LA T	L40	12	23	7	2	13	24	6	4	11	13	13	5
Ring LA T	L45	15	13	24	29	31	23	34	29	16	16	17	9
Ring LA T	L49	8	6	13	6	12	25	11	9	7	14	3	3
Ring LA T	L80	3	12	28	13	16	21	15	4	8	16	0	2

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
Burd 1 + Ring	L10	9	8	89	102	60	58	73	73	44	35	35	20
Burd 1 + Ring	L106	9	15	46	68	79	50	39	39	20	10	14	12
Burd 1 + Ring	L119	15	28	53	49	50	56	57	52	23	19	15	9
Burd 1 + Ring	L142	9	9	30	37	34	35	16	19	10	8	19	6
Burd 1 + Ring	L157	7	14	46	68	82	41	41	35	3	16	12	19
Burd 1 + Ring	L173	24	21	52	65	67	59	23	26	19	11	15	15
Burd 1 + Ring	L5	8	10	90	93	129	136	136	102	78	75	70	64
Burd 1 + Ring	L66	15	15	29	49	34	52	61	26	16	9	8	14
Burd 1 + Ring	L77	7	13	65	103	102	71	78	43	52	44	23	15
Burd 5 + Ring	L109	15	15	49	44	66	108	57	23	12	16	23	17
Burd 5 + Ring	L126	13	30	66	67	60	58	58	56	21	12	10	6
Burd 5 + Ring	L140	3	2	42	58	51	41	36	45	32	43	37	14
Burd 5 + Ring	L146	0	27	65	78	67	63	35	10	2	0	0	0
Burd 5 + Ring	L18	10	14	60	95	80	64	58	78	78	70	58	29
Burd 5 + Ring	L182	1	10	37	0	58	56	38	15	1	19	23	10
Burd 5 + Ring	L39	7	1	42	64	73	44	56	27	12	9	5	3
Burd 5 + Ring	L65	13	3	70	100	65	55	71	88	38	64	39	58
Burd 5 + Ring	L75	11	15	38	7	31	44	45	43	41	15	3	19

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
Burd 10 + Ring	L102	14	46	81	104	97	65	81	79	61	38	12	27
Burd 10 + Ring	L130	23	37	65	93	114	103	86	37	42	21	14	30
Burd 10 + Ring	L138	26	34	75	77	59	30	31	20	8	8	12	5
Burd 10 + Ring	L164	4	13	45	50	52	51	41	19	10	6	2	2
Burd 10 + Ring	L169	6	3	59	65	85	79	49	21	14	8	2	3
Burd 10 + Ring	L19	5	4	55	80	133	116	87	83	58	18	18	21
Burd 10 + Ring	L42	12	10	46	104	61	63	57	37	15	17	26	27
Burd 10 + Ring	L50	12	8	49	70	70	65	53	45	19	36	9	22
Burd 10 + Ring	L86	3	2	44	60	61	72	80	31	6	4	4	1
Burd 10 + Ring LA SC	L125	5	30	102	93	52	31	58	58	26	26	26	7
Burd 10 + Ring LA SC	L179	0	0	21	30	31	14	2	17	3	0	1	0
Burd 10 + Ring LA SC	L188	41	31	80	50	39	48	56	44	35	32	36	37
Burd 10 + Ring LA SC	L22	17	16	22	38	22	26	23	13	20	26	22	14
Burd 10 + Ring LA SC	L32	2	1	15	32	7	2	2	3	1	1	0	1
Burd 10 + Ring LA SC	L48	12	9	14	11	8	17	14	9	7	7	8	14
Burd 10 + Ring LA SC	L61	7	3	20	8	0	6	7	0	10	5	8	22
Burd 10 + Ring LA SC	L78	2	9	31	27	32	24	28	15	14	10	4	5
Burd 10 + Ring LA SC	L92	1	0	8	14	10	20	6	16	2	8	9	2

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
Burd 10 + Ring LA CD	L101	5	22	46	39	29	27	30	23	10	7	8	12
Burd 10 + Ring LA CD	L124	12	32	63	50	48	27	39	20	6	24	4	2
Burd 10 + Ring LA CD	L136	1	31	78	48	20	38	44	24	30	30	26	6
Burd 10 + Ring LA CD	L151	6	19	51	19	27	14	24	14	17	6	15	5
Burd 10 + Ring LA CD	L170	24	45	57	139	55	46	38	42	20	75	14	30
Burd 10 + Ring LA CD	L29	0	14	23	27	13	39	27	12	29	12	6	12
Burd 10 + Ring LA CD	L36	8	14	34	15	59	36	22	19	23	14	13	8
Burd 10 + Ring LA CD	L68	4	26	43	28	53	28	44	21	19	20	9	8
Burd 10 + Ring LA CD	L84	9	5	36	32	17	22	16	15	14	7	3	5
Burd 1	L112	5	8	30	22	20	17	9	20	9	21	24	13
Burd 1	L121	0	21	56	90	88	55	31	29	37	24	9	2
Burd 1	L167	0	3	38	52	31	55	53	13	7	31	6	2
Burd 1	L172	17	49	63	81	38	16	34	10	13	9	9	7
Burd 1	L31	21	5	21	30	23	8	10	2	7	2	3	19
Burd 1	L46	5	11	8	26	53	50	24	21	6	2	3	1
Burd 1	L56	6	9	31	41	14	17	29	27	17	11	7	13
Burd 1	L8	20	11	29	85	86	73	73	107	87	58	44	15
Burd 1	L81	32	39	61	115	102	111	92	29	58	29	24	17

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
Burd 10	L11	3	28	85	114	111	116	102	44	10	29	49	71
Burd 10	L111	3	10	27	27	16	26	46	16	17	23	19	9
Burd 10	L127	6	20	58	72	72	49	63	87	96	64	39	23
Burd 10	L129	21	45	68	48	21	57	85	63	27	42	30	17
Burd 10	L154	124	104	186	162	232	197	232	255	186	197	197	108
Burd 10	L174	43	45	48	36	21	39	22	17	126	86	12	23
Burd 10	L43	13	15	55	74	80	92	86	66	52	30	20	13
Burd 10	L58	31	65	37	109	102	110	206	210	122	107	190	80
Burd 10	L76	7	5	24	27	10	4	9	8	4	10	11	4
Burd 10 LA SC	L103	7	12	35	26	21	22	158	22	10	19	12	34
Burd 10 LA SC	L110	3	14	65	93	102	164	100	74	55	63	26	21
Burd 10 LA SC	L120	15	26	52	86	48	41	105	70	59	89	47	38
Burd 10 LA SC	L159	17	8	31	35	36	17	7	42	28	14	16	20
Burd 10 LA SC	L185	1	3	22	27	45	23	13	53	42	13	15	21
Burd 10 LA SC	L27	17	23	16	34	15	17	39	12	37	30	29	31
Burd 10 LA SC	L60	12	12	30	36	23	37	20	26	17	17	13	23

		TIME											
TREATMENT	**ANIMAL**	**-15**	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
Burd 10 LA CD	L122	1	29	44	42	27	13	30	52	14	15	12	10
Burd 10 LA CD	L163	4	13	45	50	52	41	20	10	6	2	2	3
Burd 10 LA CD	L17	6	19	56	46	42	29	16	14	15	28	19	5
Burd 10 LA CD	L176	1	7	46	51	78	99	74	77	101	86	49	55
Burd 10 LA CD	L28	6	31	86	75	100	65	80	103	67	56	73	56
Burd 10 LA CD	L62	21	29	55	64	79	71	79	43	34	36	16	20
Burd 10 LA CD	L74	1	4	30	26	36	12	19	22	5	29	14	29
Burd 10 LA CD	L83	15	37	28	38	12	10	15	17	16	7	29	16
Burd 10 LA CD	L1	12	15	71	60	71	44	33	41	29	32	26	20

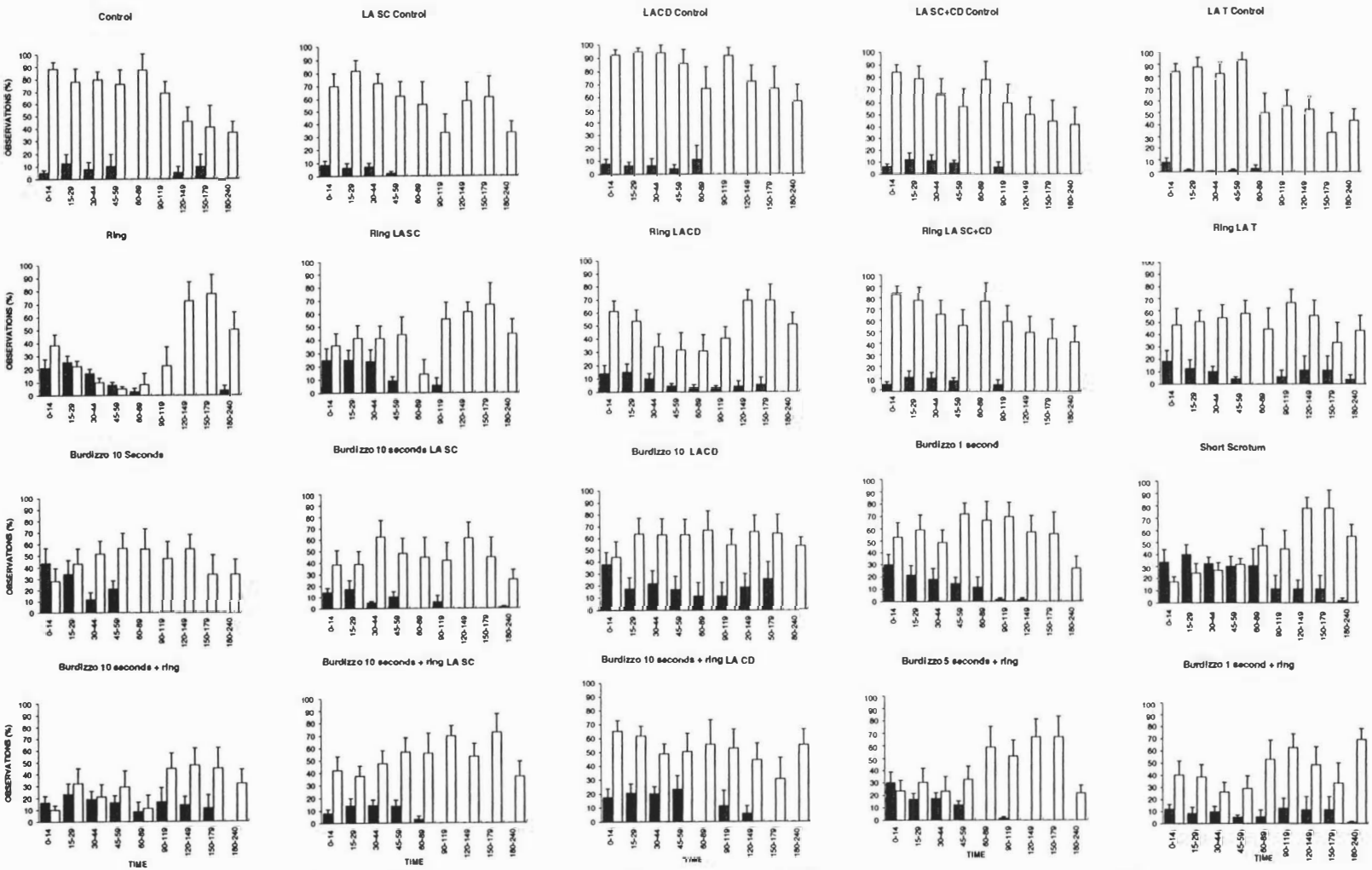
APPENDIX 2: PLASMA CORTISOL CONCENTRATIONS (CHAPTER 4).

		Time										
Treatment	Lamb	0	15	30	45	60	90	120	150	180	210	240
Ring CT	L1	42	74	90	90	80	77	61	15	44	27	38
	L4	36	32	70	114	80	37	30	11	10	15	6
	L7	29	53	23	77	64	0	71	17	20	1	9
	L10	12	82	42	59	114	85	21	10	14	2	8
	L13	28	66	74	151	104	94	45	20	35	14	13
	L16	40	64	100	139	37	94	110	20	31	52	28
	L19	15	78	65	50	0	51	31	6	12	10	0
	L22	19	88	90	107	99	66	57	15	37	31	29
	L25	26	39	97	131	86	116	127	11	5	16	2
	L28	25	74	59	151	110	167	63	13	11	27	15
Ring +	L2	57	90	104	128	109	89	31	8	5	1	4
Burd CT	L5	15	35	93	84	75	73	45	11	5	6	7
	L8	21	10	81	84	186	49	68	12	17	29	13
	L11	50	24	63	116	77	133	47	27	35	27	20
	L14	45	41	89	139	70	87	61	17	35	22	21
	L17	31	65	136	99	114	21	30	8	3	6	6
	L20	23	17	72	49	35	56	30	14	20	14	8

		Time										
Treatment	Lamb	0	15	30	45	60	90	120	150	180	210	240
Ring +	L23	24	66	115	140	111	95	68	11	2	14	6
Burd CT	L26	28	41	82	66	56	92	45	20	4	9	24
(Cont.)	L29	8	64	114	128	99	97	44	8	19	9	9
Ring +	L3	18	58	35	60	45	61	16	8	15	3	1
Burd C &	L6	29	66	70	135	86	79	54	8	4	9	10
Ring T	L9	34	80	124	171	70	140	111	19	51	23	23
	L12	38	53	96	116	128	65	56	19	23	27	27
	L15	46	56	85	93	109	101	94	26	42	32	31
	L18	33	85	108	66	46	65	28	9	10	3	10
	L21	32	31	132	138	103	70	110	20	61	80	32
	L24	13	70	78	92	102	133	117	10	2	0	16
	L27	17	72	97	104	94	142	33	11	9	5	10
	L30	32	35	77	116	118	164	54	13	13	34	14

Table A2.1 The plasma cortisol concentrations of the lambs used in Chapter 2.

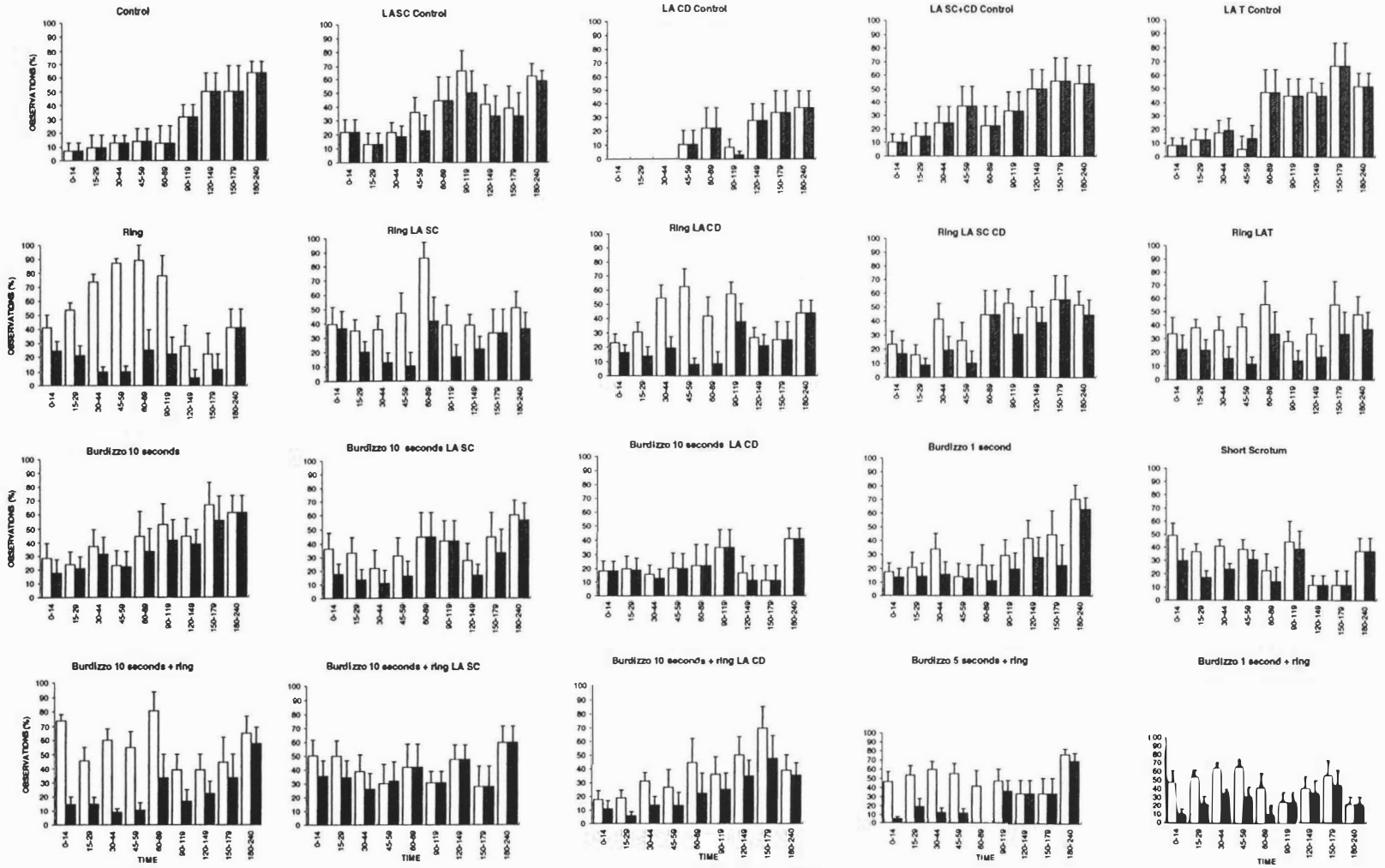
APPENDIX 3: BEHAVIOURAL DATA (CHAPTER 5).



Appendix 3 Fig. 1. The amount of normal or abnormal standing/walking, expressed as the percentage (mean ± SEM) of observations in each time period.

Abnormal Standing/Walking

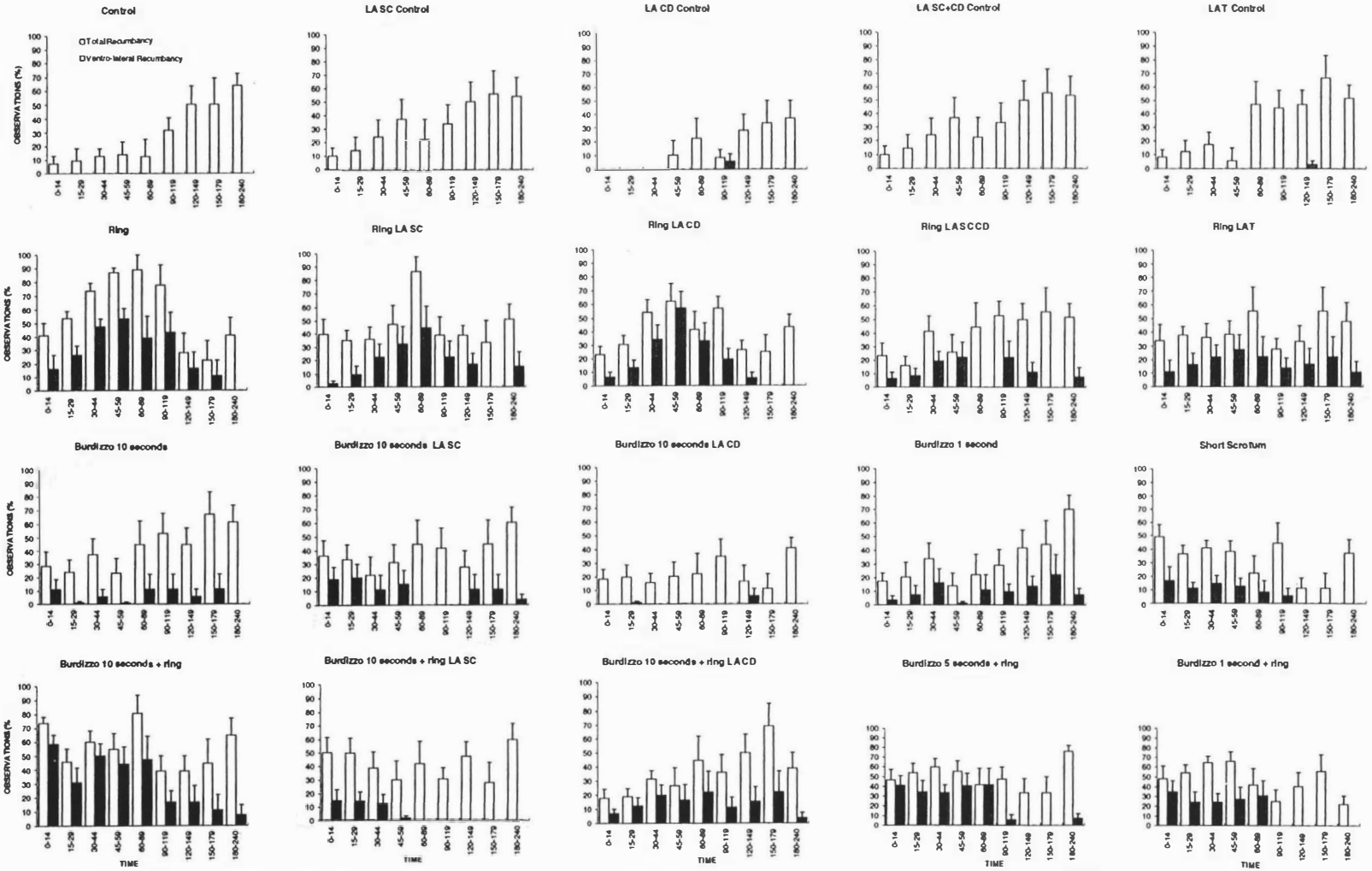
Normal Standing/Walking



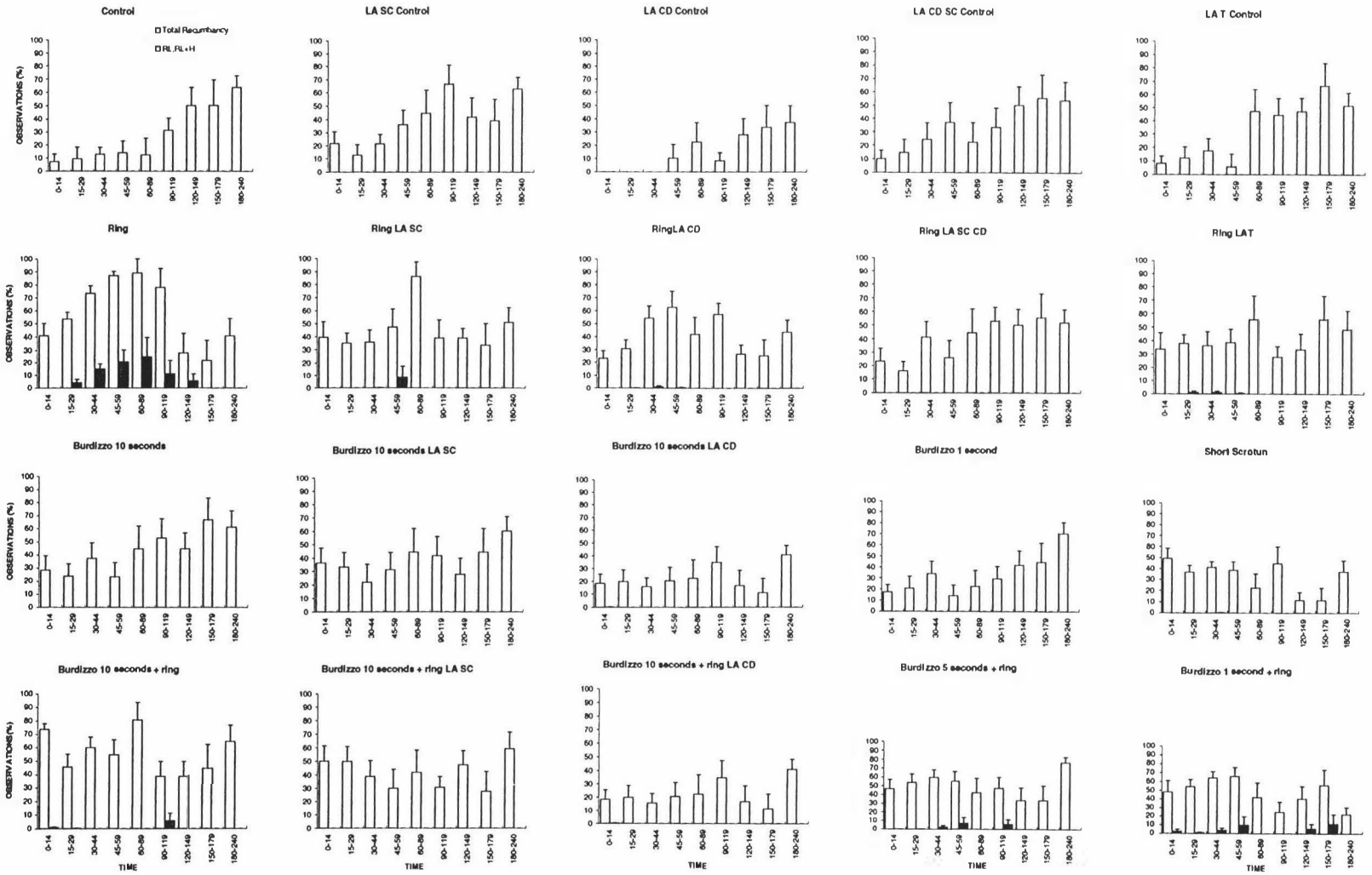
Appendix 3 Fig. 2. The amount of behaviours that were recumbant (Total Recumbancy) or ventrally recumbant, expressed as the percentage of observations (Mean ± SEM) in each time period.

Total Recumbancy

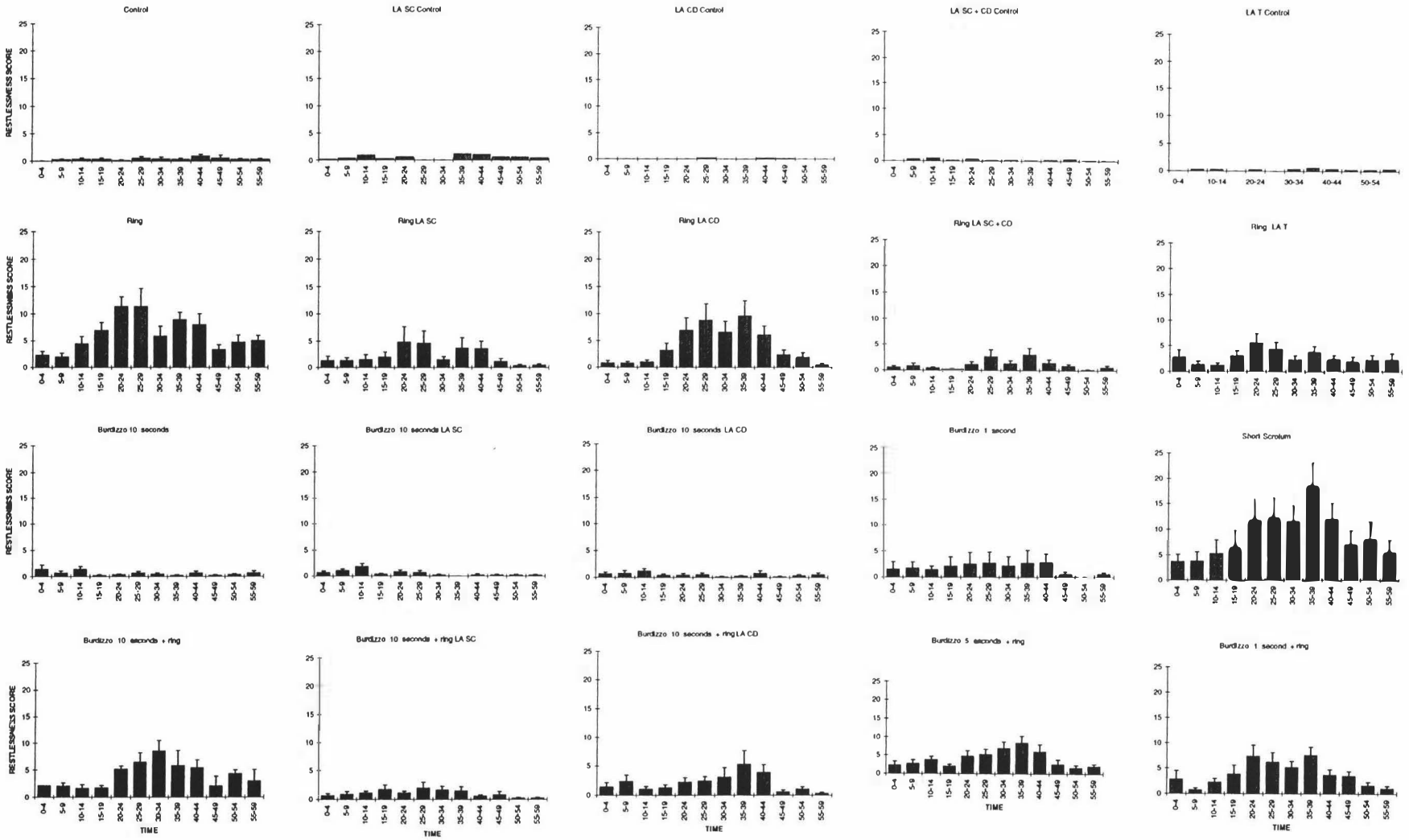
Ventral Recumbancy



Appendix 3 Fig. 3. The amounts of recumbancy (Total recumbancy) and ventro-lateral recumbancy, expressed as percentage (Mean + SEM) of observations in each time period.



Appendix 3 Fig. 4. The amount of recumbancy and lateral recumbancy observed, expressed as the percentage (mean ± SEM) of observations during each time period.



Appendix 3 Fig. 5. Restlessness scores during the first hour after different treatments.

NORMAL WALKING/STANDING (1 hour)

A=p<0.05, B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control						B	B	B	A	A	A	B	B	B	A	A		A	A	
LA SC Control			A			B	B	A	A			A	A	B	A			A		
LA CD Control		A		A		B	B	B	B	B	B	B	B	B	B	B	A	B	A	A
LA SC+CD Control			A			B	B	A	A			A	A	A						
LA T Control						B	B	B	B	A	A	B	B	B	A	A	A	A	A	
Short Scrotum	B	B	B	B	B				A	A	A				A	A	A	A	A	A
Ring	B	B	B	B	B			A	A	A	A				A	B	A	A	A	A
Ring LA SC	B	A	B	A	B		A													
Ring LA CD	A	A	B	A	B	A	A													
Ring LA SC+CD	A		B		A	A	A							A						
Ring LA T	A		B		A	A	A							A						
Burd 1 + Ring	B	A	B	A	B											A	A			
Burd 5 + Ring	B	A	B	A	B											A	A			A
Burd 10 + Ring	B	B	B	A	B					A	A					A	A		A	A
Burd 10 + Ring LA SC	A	A	B		A	A	A													
Burd 10 + Ring LA CD	A		B		A	A	B					A	A	A						
Burd 1			A		A	A	A					A	A	A						
Burd 10	A	A	B		A	A	A													
Burd 10 LA SC	A		A		A	A	A							A						
Burd 10 LA CD			A			A	A						A	A						

TABLE 1 Significant differences between amounts of normal walking or standing exhibited during 1 hour after different treatments. A=p<0.05, B=p<0.001.

NORMAL WALKING/STANDING (4 hours)

A=p<0.05 B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA	Burd 10 + Ring LA	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control						B	B	B	A	A	A	B	B	B	A	A		A	A	
LA SC Control			A			B	B	A	A			A	A	B	A			A		
LA CD Control		A		A		B	B	B	B	B	B	B	B	B	B	B	A	B	A	A
LA SC+CD Control			A			A	B	A				A	A	A						
LA T Control						B	B	B	A	A	A	B	B	B	A	A	A	A	A	
Short Scrotum	B	B	B	A	B				A	A	A					A	A			A
Ring	B	B	B	B	B			A	A	A	A				A	B	A	A	A	A
Ring LA SC	B	A	B	A	B		A													
Ring LA CD	A	A	B		A	A	A													
Ring LA SC+CD	A		B		A	A	A							A						
Ring LA T	A		B		A	A	A							A						
Burd 1 + Ring	B	A	B	A	B															
Burd 5 + Ring	B	A	B	A	B											A	A			A
Burd 10 + Ring	B	B	B	A	B					A	A					A	A			A
Burd 10 + Ring LA SC	A	A	B		A		A													
Burd 10 + Ring LA CD	A		B		A	A	B						A	A						
Burd 1			A		A	A	A						A	A						
Burd 10	A	A	B		A		A													
Burd 10 LA SC	A		A		A		A													
Burd 10 LA CD			A			A	A						A	A						

TABLE 2 Significant differences between amounts of normal walking or standing exhibited for four hours after different treatments. A = p<0.05 B = p<0.001.

ABNORMAL WALKING/STANDING (1 Hour)

A=p<0.05, B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control						A				A										
LA SC Control						A	A	A		B			A			A		A		
LA CD Control						A		A		A			A			A		A		
LA SC+CD Control						A				A										
LA T Control						A	A	A		B			A	A	A	A	A	A	A	A
Short Scrotum	A	A	A	A	A		A		A		A	A			A				A	
Ring		A			A	A														
Ring LA SC		A	A		A															
Ring LA CD						A														
Ring LA SC+CD	A	B	A	A	B							A								
Ring LA T						A														
Burd 1 + Ring						A				A										
Burd 5 + Ring		A	A		A															
Burd 10 + Ring					A															
Burd 10 + Ring LA SC					A	A														
Burd 10 + Ring LA CD		A	A		A															
Burd 1					A															
Burd 10		A	A		A															
Burd 10 LA SC					A	A														
Burd 10 LA CD					A															

TABLE 3 Significant differences between amounts of abnormal walking or standing exhibited during 1 hour after different treatments. A=p<0.05, B=p<0.001.

ABNORMAL WALKING/STANDING (4 Hours)

A=p<0.05 B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control						A				A										
LA SC Control						A	A	A		A			A			A		A		
LA CD Control						A		A		A			A			A		A		
LA SC+CD Control						A				A										
LA T Control						A	A	A		B			A	A	A	A	A	A	A	A
Short Scrotum	A	A	A	A	A				A		A	A			A				A	
Ring		A			A															
Ring LA SC		A	A		A															
Ring LA CD						A														
Ring LA SC+CD	A	A	A	A	B							A								
Ring LA T						A														
Burd 1 + Ring						A				A										
Burd 5 + Ring		A	A		A															
Burd 10 + Ring					A															
Burd 10 + Ring LA SC					A	A														
Burd 10 + Ring LA CD		A	A		A															
Burd 1					A															
Burd 10		A	A		A															
Burd 10 LA SC					A	A														
Burd 10 LA CD					A															

TABLE 4 Significant differences between amounts of abnormal walking or standing exhibited during 4 hours after different treatments. A=p<0.05, B=p<0.001.

VENTRAL RECUMBANCY (1 Hour)

A=p<0.05, B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control						A									A					
LA SC Control																				
LA CD Control						B	A	A	A			A			A			A		A
LA SC+CD Control																				
LA T Control																				
Short Scrotum	A		B										A	A		A				
Ring			A																	
Ring LA SC			A																	
Ring LA CD			A																	
Ring LA SC+CD																				
Ring LA T																				
Burd 1 + Ring			A																	
Burd 5 + Ring						A									A					
Burd 10 + Ring						A									A					
Burd 10 + Ring LA SC	A		A										A	A		A				
Burd 10 + Ring LA CD						A									A					
Burd 1																				
Burd 10			A																	
Burd 10 LA SC																				
Burd 10 LA CD			A																	

TABLE 5 Significant differences between amounts of ventral recumbency exhibited during 1 hour after different treatments. A=p<0.05, B=p<0.001.

VENTRAL RECUMBANCY (4 Hours)

A=p<0.05 B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control															A					
LA SC Control			A																	
LA CD Control		A				B	A	A	A			A	A	A	A			A		A
LA SC+CD Control																				
LA T Control																				
Short Scrotum			B													A				
Ring			A																	
Ring LA SC			A																	
Ring LA CD			A																	
Ring LA SC+CD																				
Ring LA T																				
Burd 1 + Ring			A												A					
Burd 5 + Ring			A												A					
Burd 10 + Ring																				
Burd 10 + Ring LA SC	A		A										A	A		A				
Burd 10 + Ring LA CD						A									A					
Burd 1			A																	
Burd 10			A																	
Burd 10 LA SC																				
Burd 10 LA CD			A																	

TABLE 6 Significant differences between amounts of ventral recumbency exhibited during 4 hours after different treatments. A=p<0.05, B=p<0.001.

VENTRO-LATERAL RECUMBANCY (1 hour)

A=p<0.05, B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control							B	A	A	A	A	A	A	B	A	A				
LA SC Control							B		A			A	A	B						
LA CD Control							B	A	A	A	A	A	A	B	A	A				
LA SC+CD Control							B	A	A	A	A	A	A	B	A	A				
LA T Control							B	A	A	A	A	A	A	B	A	A				
Short Scrotum							A						A	A						
Ring	B	B	B	B	B	A		A		A					A	A	A	B		B
Ring LA SC	A		A	A	A		A							A						A
Ring LA CD	A	A	A	A	A										A		A	A		A
Ring LA SC+CD	A		A	A	A		A						A	A						A
Ring LA T	A		A	A	A									A						A
Burd 1 + Ring	A	A	A	A	A												A	A		A
Burd 5 + Ring	A	A	A	A	A	A				A					A	A	A	A		A
Burd 10 + Ring	B	B	B	B	B	A		A		A	A				A	A	A	B	A	B
Burd 10 + Ring LA SC	A		A	A	A		A		A				A	A						A
Burd 10 + Ring LA CD	A		A	A	A		A						A	A						
Burd 1							A		A			A	A	A						
Burd 10							B		A			A	A	B						
Burd 10 LA SC														A						
Burd 10 LA CD							B	A	A	A	A	A	A	B	A					

TABLE 7 Significant differences between amounts of ventro-lateral recumbency exhibited during 1 hour after different treatments. A=p<0.05, B=p<0.001.

VENTRO-LATERAL RECUMBANCY (4 hours)

A=p<0.05, B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control							B	A	A	A	A	A	A	B	A	A				
LA SC Control							B		A			A	A	B						
LA CD Control							B	A	A	A	A	A	A	B	A					
LA SC+CD Control							B	A	A	A	A	A	A	B	A	A				
LA T Control							B	A	A	A	A	A	A	B	A					
Short Scrotum							A						A	A						
Ring	B	B	B	B	B	A				A					A	A	A	B		B
Ring LA SC	A		A	A	A									A						A
Ring LA CD	A	A	A	A	A										A		A	A		A
Ring LA SC+CD	A		A	A	A		A						A	A						A
Ring LA T	A		A	A	A									A						A
Burd 1 + Ring	A	A	A	A	A												A	A		A
Burd 5 + Ring	A	A	A	A	A	A				A					A	A	A	A		A
Burd 10 + Ring	B	B	B	B	B	A		A		A	A				A	A	A	B	A	B
Burd 10 + Ring LA SC	A		A	A	A		A		A				A	A						A
Burd 10 + Ring LA CD	A			A			A						A	A						
Burd 1							A		A			A	A	A						
Burd 10							B		A			A	A	B						
Burd 10 LA SC														A						
Burd 10 LA CD							B	A	A	A	A	A	A	B	A					

TABLE 8 Significant differences between amounts of ventro-lateral recumbency exhibited during 4 hours after different treatments. A=p<0.05, B=p<0.001.

LATERAL RECUMBANCY (1 hour)

A=p<0.05, B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control							A		A											
LA SC Control							A		A											
LA CD Control							A		A											
LA SC+CD Control							A		A											
LA T Control							A		A											
Short Scrotum							A													
Ring	A	A	A	A	A	A			A	A	A		A	A	A	A	A	A	A	A
Ring LA SC																				
Ring LA CD	A	A	A	A	A		A								A		A	A	A	A
Ring LA SC+CD							A													
Ring LA T							A													
Burd 1 + Ring																				
Burd 5 + Ring							A													
Burd 10 + Ring							A													
Burd 10 + Ring LA SC							A		A											
Burd 10 + Ring LA CD							A													
Burd 1							A		A											
Burd 10							A		A											
Burd 10 LA SC							A		A											
Burd 10 LA CD							A		A											

TABLE 9 Significant differences between amounts of lateral recumbency exhibited during 1 hour after different treatments. A=p<0.05, B=p<0.001.

LATERAL RECUMBANCY (4 hours)

A=p<0.05 B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control							A		A					A						
LA SC Control							A		A					A						
LA CD Control							A		A					A						
LA SC+CD Control							A		A					A						
LA T Control							A		A					A						
Short Scrotum							A													
Ring	A	A	A	A	A	A		A	A	A	A		A	A	A	A	A	A	A	A
Ring LA SC							A													
Ring LA CD	A	A	A	A	A		A								A		A	A	A	A
Ring LA SC+CD							A													
Ring LA T							A													
Burd 1 + Ring																				
Burd 5 + Ring							A													
Burd 10 + Ring	A	A	A	A	A		A								A		A	A	A	A
Burd 10 + Ring LA SC							A		A					A						
Burd 10 + Ring LA CD							A													
Burd 1							A		A					A						
Burd 10							A		A					A						
Burd 10 LA SC							A		A					A						
Burd 10 LA CD							A		A					A						

TABLE 10 Significant differences between amounts of lateral recumbency exhibited during 1 hour after different treatments. A=p<0.05, B=p<0.001.

TOTAL RECUMBANCY (1 hour)

A=p<0.05, B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control						A	B	A	A	A	A	B	B	B	A					
LA SC Control			A			A	B		A			A	A	A						
LA CD Control		A				B	B	B	B	A	B	B	B	B	A	A	A	A	A	A
LA SC+CD Control							B					A	A	A						
LA T Control						A	B	A	A		A	B	B	B	A					
Short Scrotum	A	A	B		A		A					A				A	A			A
Ring	B	B	B	B	B	A		A	A	B	A				A	B	B	B	A	B
Ring LA SC	A		B		A		A					A								A
Ring LA CD	A	A	B		A		A									A	A			A
Ring LA SC+CD	A		A				B					A	A	A						
Ring LA T	A		B		A		A					A		A						A
Burd 1 + Ring	B	A	B	A	B	A		A		A	A					B	B	A		B
Burd 5 + Ring	B	A	B	A	B					A						A	A	A		B
Burd 10 + Ring	B	A	B	A	B					A	A					A	A	A		B
Burd 10 + Ring LA SC	A		A		A		A													A
Burd 10 + Ring LA CD			A			A	B		A			B	A	A						
Burd 1			A			A	B		A			B	A	A						
Burd 10			A				B					A	A	A						
Burd 10 LA SC			A				A													
Burd 10 LA CD			A			A	B	A	A		A	B	B	B	A					

TABLE 11 Significant differences between amounts of recumbent behaviour exhibited during 1 hour after different treatments. A=p<0.05, B=p<0.001.

TOTAL RECUMBANCY (4 hour)

A=p<0.05 B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control		A	A	A	B	A		B	B	A		B	A							A
LA SC Control	A										A	A		A		A		B	A	
LA CD Control	A				A			A	A			B						A		
LA SC+CD Control	A								A			A		A		A		B		
LA T Control	B		A				A				B			B		B	A	B	A	A
Short Scrotum	A								A			A		A		A		B		
Ring					A			A	A			B						A		
Ring LA SC	B		A				A				A			B		B	A	B	A	A
Ring LA CD	B		A	A		A	A				B		A	B	A	B	A	B	A	A
Ring LA SC+CD	A													A		A		A		
Ring LA T		A			B			A	B			B						A		
Burd 1 + Ring	B	A	B	A		A	B				B		A	B	A	B	A	B	B	B
Burd 5 + Ring	A								A			A		A		A		B		
Burd 10 + Ring		A		A	B	A		B	B	A		B	A							
Burd 10 + Ring LA SC									A			A						A		
Burd 10 + Ring LA CD		A		A	B	A		B	B	A		B	A					A		
Burd 1					A			A	A			A								
Burd 10		B	A	B	B	B	A	B	B	A	A	B	B		A	A			A	A
Burd 10 LA SC		A			A			A	A			B						A		
Burd 10 LA CD	A				A			A	A			B						A		

TABLE 12 Significant differences between amounts of recumbent behaviour exhibited during 4 hours after different treatments. A=p<0.05, B=p<0.001.

TOTAL ABNORMAL RECUMBANCY (1 hour)

A=p<0.05, B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 1	Burd 10 LA SC	Burd 10 LA CD
Control						A	B	A	A	A	A	A	A	B	A	A				
LA SC Control							B		A			A	A	B						
LA CD Control						A	B	A	A	A	A	A	A	B	A	A				
LA SC+CD Control						A	B	A	A	A	A	A	A	B	A	A				
LA T Control						A	B	A	A	A	A	A	A	B	A	A				
Short Scrotum	A		A	A	A		A						A	A						A
Ring	B	B	B	B	B	A		A		B	A				B	A	B	B	A	B
Ring LA SC	A		A	A	A		A							A						A
Ring LA CD	A	A	A	A	A										A		A	A		A
Ring LA SC+CD	A		A	A	A		B						A	A						A
Ring LA T	A		A	A	A		A							A						A
Burd 1 + Ring	A	A	A	A	A										A		A	A		A
Burd 5 + Ring	A	A	A	A	A	A				A					A	A	A	A		A
Burd 1 + Ring	B	B	B	B	B	A		A		A	A				A	A	A	B		B
Burd 1 + Ring LA SC	A		A	A	A		B		A			A	A	A						A
Burd 1 + Ring LA CD	A		A	A	A		A						A	A						A
Burd 1							B		A			A	A	A						
Burd 1							B		A			A	A	B						
Burd 1 LA SC							A													
Burd 1 LA CD						A	B	A	A	A	A	A	A	B	A	A				

TABLE 13 Significant differences between amounts of abnormal recumbent behaviour exhibited during 1 hour after different treatments. A=p<0.05, B=p<0.001.

TOTAL ABNORMAL RECUMBANCY (4 hours)

A=p<0.05 B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control						A	B	A	A	A	A	A	A	B	A	A				
LA SC Control							B		A			A	A	B						
LA CD Control						A	B	A	A	A	A	A	A	B	A	A				
LA SC+CD Control						A	B	A	A	A	A	A	A	B	A	A				
LA T Control						A	B	A	A	A	A	A	A	B	A	A				
Short Scrotum	A		A	A	A		A						A	A						A
Ring	B	B	B	B	B	A		A	A	A	A				B	A	B	B	A	B
Ring LA SC	A		A	A	A		A							A						A
Ring LA CD	A	A	A	A	A		A								A		A	A		A
Ring LA SC+CD	A		A	A	A		A						A	A						A
Ring LA T	A		A	A	A		A							A						A
Burd 1 + Ring	A	A	A	A	A										A		A	A		A
Burd 5 + Ring	A	A	A	A	A	A				A					A	A	A	A		A
Burd 10 + Ring	B	B	B	B	B	A		A		A	A				A	A	A	B	A	B
Burd 10 + Ring LA SC	A		A	A	A		B		A			A	A	A						A
Burd 10 + Ring LA CD	A		A	A	A		A						A	A						A
Burd 1							B		A			A	A	A						
Burd 10							B		A			A	A	B						
Burd 10 LA SC							A							A						
Burd 10 LA CD						A	B	A	A	A	A	A	A	B	A	A				

TABLE 14 Significant differences between amounts of abnormal recumbent behaviour exhibited during 4 hours after different treatments. A=p<0.05, B=p<0.001.

TOTAL ABNORMAL BEHAVIOUR (1 hour)

A=p<0.05 B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control						A				A										
LA SC Control						A	A	A		B			A			A		A		
LA CD Control						A		A		A			A			A		A		
LA SC+CD Control						A				A										
LA T Control						A	A	A		B			A	A	A	A	A	A	A	A
Short Scrotum	A	A	A	A	A		A		A		A	A			A				A	
Ring		A			A	A														
Ring LA SC		A	A		A															
Ring LA CD						A														
Ring LA SC+CD	A	B	A	A	B							A								
Ring LA T						A														
Burd 1 + Ring						A				A										
Burd 5 + Ring		A	A		A															
Burd 10 + Ring					A															
Burd 10 + Ring LA SC					A	A														
Burd 10 + Ring LA CD		A	A		A															
Burd 1					A															
Burd 10		A	A		A															
Burd 10 LA SC					A	A														
Burd 10 LA CD					A															

TABLE 15 Significant differences between amounts of abnormal behaviour exhibited during 1 hour after different treatments. A=p<0.05, B=p<0.001.

TOTAL ABNORMAL BEHAVIOUR (4 hours)

A=p<0.05 B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control						B	B	A	A	A	A	A	B	B		A	A	A		
LA SC Control						B	B	A	A	B		A	B	B		A	A	A		
LA CD Control						B	B	A	B	B	A	A	B	B	A	A	A	A		
LA SC+CD Control						B	B	A	A	B	A	A	B	B		A	A	A		
LA T Control						B	B	A	B	B	A	A	B	B	A	B	A	A	A	A
Short Scrotum	B	B	B	B	B										A		A			A
Ring	B	B	B	B	B				A	A	A				B	A	B	A	A	A
Ring LA SC	A	A	A	A	A															
Ring LA CD	A	A	B	A	B		A								A					
Ring LA SC+CD	A	B	B	B	B		A							A						
Ring LA T	A		A	A	A		A							A						
Burd 1 + Ring	A	A	A	A	A															
Burd 5 + Ring	B	B	B	B	B										A		A	A	A	A
Burd 10 + Ring	B	B	B	B	B					A	A				A	A	A	A	A	A
Burd 10 + Ring LA SC			A		A	A	B		A				A	A						
Burd 10 + Ring LA CD	A	A	A	A	B		A							A						
Burd 1	A	A	A	A	A	A	B						A	A						
Burd 10	A	A	A	A	A		A						A	A						
Burd 10 LA SC					A		A						A	A						
Burd 10 LA CD					A	A	A						A	A						

TABLE 16 Significant differences between amounts of abnormal behaviour exhibited during 4 hours after different treatments. A=p<0.05, B=p<0.001.

RESTLESSNESS SCORE (1 hour)

A=p<0.05 B=p<0.001	Control	LA SC Control	LA CD Control	LA SC+CD Control	LA T Control	Short Scrotum	Ring	Ring LA SC	Ring LA CD	Ring LA SC+CD	Ring LA T	Burd 1 + Ring	Burd 5 + Ring	Burd 10 + Ring	Burd 10 + Ring LA SC	Burd 10 + Ring LA CD	Burd 1	Burd 10	Burd 10 LA SC	Burd 10 LA CD
Control						A	B		A		A	B	A	A	A	A				
LA SC Control			A			A	B		A		A	A	A	A		A				
LA CD Control		A				A	B	A	A	A	A	B	A	A	A	A		A	A	A
LA SC+CD Control						A	B		A	A	A	B	A	A	A	A				
LA T Control						A	B		A	A	A	B	A	A	A	A				
Short Scrotum	A	A	A	A	A			A		A					A	A	A	A	A	A
Ring	B	B	B	B	B			A		B	A				B	A	B	B	B	B
Ring LA SC			A			A	A													
Ring LA CD	A	A	A	A	A					A					A		A	A	A	A
Ring LA SC+CD			A	A	A	A	B		A			A	A	A						
Ring LA T	A	A	A	A	A		A										A	A	A	A
Burd 1 + Ring	B	A	B	B	B					A					A		B	A	A	A
Burd 5 + Ring	A	A	A	A	A					A					A		A	A	A	A
Burd 10 + Ring	A	A	A	A	A					A					A		A	A	A	A
Burd 10 + Ring LA SC	A		A	A	A	A	B		A			A	A	A			A			
Burd 10 + Ring LA CD	A	A	A	A	A	A	A										A	A	A	A
Burd 1						A	B		A		A	B	A	A	A	A				
Burd 10			A			A	B		A		A	A	A	A		A				
Burd 10 LA SC			A			A	B		A		A	A	A	A		A				
Burd 10 LA CD			A			A	B		A		A	A	A	A		A				

TABLE 17 Significant differences between total restlessness scores recorded during 1 hour after different treatments. A=p<0.05, B=p<0.001.

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