The safety and efficacy of intramuscular xylazine for pain relief in sheep and lambs

Cliff Grant

Department of Anaesthesia and Intensive Care
Faculty of Medicine
Division of Health Sciences
University of Adelaide,
Adelaide, S.A. 5005
Australia

Thesis submitted for the Degree of Master of Medical Science

April 2002

Contents

List of Tables

List of Figures

Abstract

Painful husbandry and surgical procedures are performed on millions of sheep each year, yet the use of analgesics to manage this pain is limited. In Chapter 1 previous studies of analgesics in sheep, and their methodology, were reviewed. It was concluded that the provision of analgesia to sheep is complicated by species specific differences that render many analgesics ineffective in the sheep. However, it was noted that relatively low doses of the α_2 adrenoceptor agonist xylazine could reduce nociception to painful mechanical, electrical and thermal stimulus in sheep. This thesis therefore examined the suitability of xylazine for providing safe and effective analgesia in two settings: for post-surgical pain in adult sheep used in biomedical research, and for routine husbandry procedures applied to lambs on farms. In Australia, the latter setting includes the process of the Mulseing - the removal of skin around the anus and hock to prevent fly strike - in addition to the standard practices of tail-docking and castration using rubber rings or a hot knife. With respect to the first setting, in Chapter 3 the effect of the administration route of a single dose of xylazine (2.5mg) on the resultant analgesic profile (deduced from the threshold electrical current needed to elicit a leg withdrawal response) was examined. The intravenous, intramuscular and subcutaneous routes produced peak analgesia at approximately 15, 25 and 30 min respectively. It was concluded that intramuscular administration of xylazine was simple

to perform yet was characterised by a rapid peak analgesic effect with a reasonable duration of action. In Chapter 4, the cardiovascular effects on an analgesic dose of intramuscular xylazine were measured. It was shown that this dose had minimal deleterious effects on cardiac output, blood pressure or arterial blood gases. In Chapter 5, the data provided by the first two chapters was used to develop and validate an intravenous infusion regimen to provide continuous analgesia in adult sheep. The remaining chapters addressed the use of xylazine in the second setting. In Chapter 6 it was shown that the anti-nociceptive effects of xylazine in lambs (4–6 weeks) were of a similar magnitude and duration to those in adult sheep when the dose was scaled for body weight. Thus, there was no evidence of analgesic differences due to developmental or age related factors. In Chapter 7, existing behavioural measures for assessing pain in lambs were validated using discriminant analysis against a range of husbandry procedures, including Mulseing. From this a ranking of the relative painfulness of different husbandry procedures was developed. In Chapter 8, these validated behavioural measures were used to assess the efficacy of intramuscular xylazine. It was shown that xylazine reduced the pain response from surgical husbandry procedures such as Mulesing, but was ineffective in treating the pain of husbandry treatments that used tight rubber rings. In summary, the studies from this thesis demonstrated that intramuscular administration of low dose xylazine provided safe and predictable analgesia suitable for the treatment of surgical pain, with comparable analgesic effects between adult sheep and lambs. This provides support for further investigation into the use of α_2 agonists for the treatment of this type of pain in sheep and lambs.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, it contains no material previously published or written by any other person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being available for loan and photocopying.

Cliff Grant
16 April 2002

Acknowledgements

This thesis has been made possible by the input and support of many people, I should like to highlight the contribution made by just a select few.

Richard Upton who initially encouraged my undertaking post-graduate study and was duly rewarded with the onerous task of being my supervisor. He has proven to be the ideal supervisor and academic mentor, with a patience seen only in the saintly or heavily sedated.

The worlds' busiest man, Tim Kuchel, a font of knowledge regarding animal pain whose enthusiasm for the subject is contagious. His encouragement and assistance in even the most minor aspects of a project enabled a smooth progression toward the completion of this thesis.

Unparalleled technical assistance from Allison Martinez and Glenda Summersides who assisted me through some of the dullest studies known to man, yet remained cheerful.

Guy Ludbrook who provided support, resources, encouragement and aimless chat far beyond the imaginable scope for any head of department. David Doolette, who would expect no thanks for his efforts and is probably right. The renaissance scientist, Ted Mayther, who was always there.

Finally and most importantly, wife Judi and children Liam, Alexander and Patrick. Who always provide great support to me in my endeavours.

Publications produced during candidacy

1. Grant, C., Summersides, G.E. & Kuchel, T.R. (2001) A xylazine infusion regimen to provide anlagesia in sheep. *Laboratory Animals* **35(3):** 277–281.
2. Grant, C. & Upton, R.N. (2001) Cardiovascular and haemodynamic effects of intramuscular doses of xylazine in conscious sheep. *Australian Veterinary Journal* **79(1):**58–60.
3. Grant, C. & Upton, R.N. (2001) The anti-nociceptive effecacy of low dose intramuscular xylazine in lambs. *Research in Veterinary Science* **70(1):**47–50.
4. Myburgh, J.A., Upton, R.N., Grant, C. & Martinez, A.M. (2001) Epinephrine, norepinephrine and dopamine infusions decrease propofol concentrations during continuous propofol infusion in an ovine model. *Intensive Care Medicine* **27(1):**276–282.
5. Upton, R.N., Ludbrook, G.L., Grant, C. & Martinez, A. (2001) In vivo cerebral pharmacokinetics and pharmacodynamics of diazepam and midazolam after short intravenous infusion administration in sheep.

Journal of Pharmacokinetics and Pharmacodynamics **28(2):**129–153.

6. Upton, R.N., Ludbrook, G.L. & Grant, C. (2001) The cerebral and systemic kinetics of thiopentone and propofol in halothane anaesthetized sheep. *Anaesthesia and Intensive Care* **29(2):**117–123.
7. Upton, R.N., Zheng, D.A., Grant, C. & Martinez, A.M. (2000) Development and validation of a recirculatory physiological model of the myocardial concentrations of lignocaine after intravenous administration in sheep. *Journal of Pharmacy and Pharmaco*logy **52(2):**181–189.
8. Upton, R.N., Ludbrook, G.L., Grant, C. & Doolette, D.J. (2000) The effect of altered cerebral blood flow on the cerebral kinetics of thiopental and propofol in sheep. *Anesthesiology* **93(4):**1085–1094.
9. Doolette, D.J., Upton, R.N. & Grant, C. (1999) Agreement between ultrasonic Doppler venous outflow and Kety and Schmidt estimates of cerebral blood flow. *Clinical and Experimental Pharmacology and Physiology* **26(9):**736–740.
10. Ludbrook, G.L., Upton, R.N., Grant, C. & Martinez, A. (1999) Prolonged dysequilibrium between blood and brain concentrations of propofol during infusions in sheep. *Acta Anaesthesiologica Scandinavica* **43(2):** 206–211.
11. Ludbrook, G.L., Upton, R.N., Grant, C. & Martinez, A. (1999) A compartmental analysis of the pharmacokinetics of propofol in sheep. *Journal of Pharmacokinetics and Biopharmaceutics* **27(3):**329–338.
12. Upton, R.N., Ludbrook, G.L., Grant, C. & Martinez, A.M. (1999) Cardiac

output is a determinant of the initial concentrations of propofol after short-infusion administration. *Anesthesia and Analgesia* **89(3):**545–552.

Abbreviations

APB Active pain behaviour

CO cardiac output

HK ~~k~~hot knife tail docking

HR heart rate

IM intramuscular

IV intravenous

MABP mean arterial blood pressure

PaO$_2$ partial pressure of oxygen in arterial blood

PaCO$_2$ partial pressure of carbon dioxide in arterial blood

RRC rubber ring castration

RRT rubber ring tail docking

SC subcutaneous

SD standard deviation

SEM standard error of the mean

Chapter 1

Review of studies examining analgesic efficacy in sheep.

1.1 Introduction

There is an growing awareness of animal welfare issues not only by animal welfare advocates but also the general public (Anonymous, 2001; Stevenson, 1994). Although increasing efforts are being made to create positive welfare environments for animals used in primary production and medical research environments, there are still many improvements to be made. For sheep, two major areas of concern are the pain associated with routine husbandry or lamb 'marking' procedures on farms, and the management of post-operative pain in sheep used for biomedical research.

1.1.1 Routine husbandry procedures in primary production

Each year in Australia approximately 30 million lambs at 4–12 weeks of age undergo painful routine husbandry procedures such as mulesing, tail docking and castration. Tail docking involves removal of the tail below the third palpable joint either by knife or application of a tight rubber ring. The testicles are removed by either cutting off one third of the bottom of the scrotum and extracting the testicles one at a time using a 'lamb marking knife' or application of a tight rubber ring at the neck of the scrotum. During the mulesing procedure, which is unique to Australia, hand shears are used to strip the skin from the base of the tail to points on both sides between the anus and the hock, an area of approximately 80cm^2. These procedures have all been described as painful to sheep and the stress response to mulesing, castration and tail docking has been well documented (Blackshaw, 1986; Shutt et al., 1988a).

These procedures are highly effective in preventing problems such as wool stain and flystrike (Watts et al., 1979; French et al., 1994) and thus are essential to the wool industry. However, these husbandry practices have received considerable public attention in recent years and there are claims that the production of wool and sheep meat comes at an unacceptable welfare cost (Townsend, 1985; Anonymous, 2001). There are concerns within the wool and sheep meat industries that consumers and international markets will choose alternative products created in environments which are perceived to be 'cruelty free' (O'Flynn and O'Dea, 1996).

Efforts have been made to develop alternative methods for these procedures which could reduce the pain induced by surgical methods. These have included replacement mulesing techniques, such as depilating the breech area using high-energy electrons (Sorell et al., 1990) or the application of a quartenary ammonia compound (Chapman et al., 1994). The efficacy of intra-testicular injection of formaldehyde to sterilise rams has also been evaluated (Mercy et al., 1985). None of these methods has been adopted, as they have proved to be less effective and untenable in general farming practice.

For any regimen to be adopted widely, it needs to be both practical and inexpensive. A regimen that is uneconomic or greatly increases the burden associated with routine husbandry procedures will have difficulty in being accepted. The surgical methods are undeniably effective and at this time are the most effective solution to preventing problems such as flystrike. A simple solution would appear to be combining the existing surgical procedures with the use of analgesics. However, analgesics have not been widely adopted for controlling pain in sheep within the farming environment.

1.1.2 Use of sheep in biomedical research

Welfare issues related to the post-operative care of experimental sheep are also under great scrutiny. Animal welfare committees and animal advocates require suitable pain management procedures be in place for the treatment of pain following surgical procedures (National Health and Medical Research Council, 1995). Apart from these ethical and legislative imperatives, the validity of any data acquired from animals suffering pain that is a result of prior surgical preparation and is unrelated to the experimental procedure must also be questioned (National Research Council, 1992). Appropriate post-operative pain control is obviously fundamental. Unfortunately for researchers using sheep, existing analgesic recommendations suffer from a lack of species specific data and are generally based upon evidence of efficacy in other species and remain untested in the sheep.

1.1.3 Analgesics and sheep

There are many analgesic agents available that could be used to treat pain in sheep following surgical procedures in either the farming or research environment. Whilst there have been a number of recommendations for pain control in sheep such as buprenorphine, codeine, pethidine, morphine or paracetamol (Flecknell, 1987; Hall and Clarke, 1983), analgesics have not been widely adopted for controlling pain in sheep, particularly within the farming environment. Even within the research environment often the wrong or no analgesic is administered. This stems from both a significant degree of interspecies variation in drug effects (Baggot, 1992) and the lack of available data on effective analgesic agents in adult sheep and lambs.

Another problem is often the inference that the impassive, placid nature of the sheep is an indication that the sheep is not experiencing pain or that a given analgesic regimen is effective. This, however is often merely a manifestiation of the limited range of behavioural indicators of stress in sheep (Kilgour and Dalton, 1984).

Xylazine, an α_2 adrenoceptor agonist, has been identified as an effective analgesic agent in sheep following low dose intramuscular administration in sheep (Grant et al., 1996). Unfortunately, previous studies have shown side effects that may be detrimental for its routine use in sheep. These include, sedation and cardiovascular and respiratory depression (Klide et al., 1975; Waterman et al., 1987; Celly et al., 1997). However, these negative effects are generally seen following higher doses or intravenous administration. Grant et al. (1996) noted no sedation following low dose intramuscular injection of xylazine but the cardiovascular and respiratory effects of this administration regimen still remain unknown.

The development of an optimal analgesic regimen for sheep based upon low dose xylazine administration requires an understanding of the degree and duration of analgesic effects following various administration methods. The cardio-pulmonary safety of this dose of xylazine also needs to be validated.

These findings can then be used to develop analgesic regimens for the control of pain in sheep following experimental and routine husbandry surgical procedures. Finally, these regimens need to demonstrate analgesic efficacy and positive welfare benefits for the sheep in "real world" settings. Only then can universal guidelines for the effective control of pain in sheep be established.

1.2 Methods available for assessing analgesic efficacy in sheep

In the following sections of this chapter, the published data examining analgesics for pain control in sheep will be discussed. The methods used to collect these data will also be discussed with a view to highlighting practical approaches for assessing the analgesic efficacy of any drug administration regimen. There are four main classes of drug which have been examined in detail: Local anaesthetics, Non-Steroidal Anti-Inflammatories (NSAID's), opioids and α_2 agonists. Particular emphasis will be placed on those with proven measures of efficacy and simple methods of administration.

Quantifying the efficacy of an analgesic presents difficulties as objective measures of pain, and hence pain reduction, are difficult to obtain. In man, pain is a sensory and emotional experience that given a set of stimulus parameters can elicit many different descriptions of the sensory experience between different human subjects (eg. throbbing, stabbing, burning).

The situation is further complicated in animals as it is not possible to obtain verbal reports of the experience. Whether animals experience pain with the same qualitative and affective responses as humans is unknown. Therefore, most researchers consider a stimulus is painful in an animal if :

1. it also produces pain in humans,
2. it approaches or exceeds a level that will damage tissue,
3. it produces escape behaviour in animals.

The following section will describe methods available for determining objective indexes of pain or pain reduction indicative of analgesia in animals.

1.2.1 Endocrinological indicators of pain

Stress, including pain, produces an endocrinological response. Cortisol is the major glucocorticoid hormone released in response to stress and the most widely used hormonal indicator of stress. It has been used to assess the adverse effects of a variety of stressful procedures in sheep including: tail docking (Kent et al., 1993; Graham et al., 1997), electroimmobilisation (Jephcott et al., 1987, 1988) castration (Mellor and Murray, 1989a) and Mulesing (Fell and Shutt, 1989; Chapman et al., 1994).

Beta-endorphin, an endogenous opioid released in response to pain has not been as widely used as an experimental index of stress, but studies in sheep have shown a release profile similar to cortisol in response to stressors such as Mulesing (Shutt et al., 1987), shearing (Jephcott et al., 1987) and restraint (Shutt et al., 1988b).

The measurement of systemic hormones, in particular cortisol, has been used to quantify the stress of many husbandry procedures in sheep. There are limitations in its use however, and great care must be taken when designing experiments and interpreting results. The relationship between plasma corticosteroid levels and the degree of stress is not necessarily linear

(Rushen, 1986b) and occasional discrepancies between cortisol levels and other measures of pain highlight these issues (Wood et al., 1991). As an example, a series of seven different husbandry procedures performed in sheep were ranked in order of severity based on alternative independent indices. Plasma cortisols were only able to find three distinct different treatments whereas behavioural indicators distinguished between 6 out of the 7 treatments (Molony and Kent, 1997).

Also, as a purely objective index of pain cortisol response suffers in that it is an index not necessarily of pain but of stress and there are many stressful non-painful stimuli that can influence these hormones, including isolation or removal from flock mates (McNatty et al., 1972). Handling or the act of taking the sample itself can cause stress and elevation of cortisol, however, this can be reduced somewhat by the use of indwelling cannulae or by taking saliva samples (Fell et al., 1985). Furthermore, large sample volumes (up to 10 ml) need to be taken for assay purposes which reduces the frequency of sampling times and hence the delineation of the stress response.

There are other factors that can also influence cortisol levels such as diurnal variations (McNatty et al., 1972) and changes during oestrus. Many drugs such as the α_2 agonists also interfere with cortisol responses (Masal et al., 1985), making it an unsuitable measure in many studies involving some form of pharmacological intervention.

1.2.2 Electrical activity of the brain

1.2.2.1 The electroencephalogram (EEG)

The EEG records cerebral post-synaptic potentials across the brain. Fourier transformation reduces these signals to their component wavelengths.

Changes in certain bandwidths are indicative of cerebral response to many different phenomenon- for instance the relationship between increase in slower frequencies and depth of anaesthesia is well established. So far, only a small number of studies have examined the relationship between changes in EEG frequency spectra and pain. The general pattern in human studies is a decrease of alpha, and an increase of beta activity with pain, although one study has shown an augmentation of alpha activity in response to pain (Bromm and Lorenz, 1998). Techniques have been developed to examine similar phenomenon in sheep. Using an escape avoidance model in response to an electrical stimulus applied to the forelimb (Ong et al., 1997) showed a correlation between absolute power in alpha, delta2 wavelengths and stimulus intensity. However, the stimulus was brief (20 ms) and the changes were only evident for a single 4s second epoch after the stimulus. Movement also meant that much data had to be excluded, particularly in the low delta frequencies.

1.2.2.2 Evoked potentials

Peripheral stimuli produce afferent electrical signals that ascend through the brainstem. If the stimuli are given at known times, succeeding electical signals can be superimposed upon one another. From this, the recurring wave form of the evoked response (ERP) emerges from the blur of background noise. The measurement of ERP's in sheep following brief (20 ms) painful electrical stimulation to the forelimb showed a stimulus dependent increases in the amplitudes of all peaks and troughs between 0.1 and 0.4 seconds (Morris et al., 1997).

1.2.2.3 Electrical activity of the brain summarry

EEG and ERP are very effective at measuring gross measures of brain function such as sedation and anaesthesia and hold much promise as methods for measuring continuous levels of stress in unrestrained animals. Whilst they appear to effectively describe the simple models of very acute pain in sheep reviewed above, their usefulness in characterizing short term to chronic pain remains unknown. Further technical developments and elucidation of appropriate EEG bandwidths indicative of pain need to be made. This becomes evident when these EEG techniques are used to measure pain from the far more complex environment of husbandry procedures (Jongman et al., 2000). Interestingly, the inference from these EEG studies is that handling of sheep may be more stressful than Mulesing and that pain related changes in EEG following castration and tail docking return to baseline within 15 minutes. In the long term ERP's may offer greater potential as a measure of pain over EEG. The existing literature suggests that they are better at extracting pain specific information from the brain than the use of EEG where specific pain related frequency bandwidths need to be isolated from the many brain activities not related to pain occurring at the same time (Bromm and Lorenz, 1998). ERPs offer a method of data reduction and a response that is simpler and easier to interpret (Scott, 1976).

1.2.3 Algesimetry methods for determining analgesic efficacy

Another method for determining the efficacy of a given analgesic is by the observation of behavioural responses to artificially applied noxious stimuli. These methods are known collectively as algesimetry, and rely upon an alteration of responses to noxious stimuli as a result of pharmacological blockade of specific pain pathways. Algesimetry methods can be classified according to the types of stimuli and responses utilised.

1.2.3.1 Algesimetry stimulus types

There are a number of stimuli capable of eliciting pain, the most important requirements of the ideal stimulus, as proposed by Beecher (1957) and others, are:

1. The stimulus should be quantifiable and controlled with precision in order to minimize variability in experimental results.
2. The stimulus would activate solely pain receptors and pathways rather than systems not specifically involved with pain, such as touch or vibration.
3. The stimulus should be repeatable and produce no tissue damage so the effects can be examined over a number of presentations.
4. The stimulus type should produce the type of pain most relevant to the researcher and be appropriate for the particular experimental circumstances. For example, a stimulus that requires restraint or sedation is obviously unsuitable for examining behavioural response following administration of an analgesic.

Thermal Thermal stimulus of the skin has been widely employed in experimental studies, particularly in the rodent. The stimulus source can be either radiant heat, as in the tail flick response, or conductive heat, as in the hot plate method. An adaptation of the above methods has been developed for use in the sheep. A small ear clip containing a heating element and a sensing thermocouple is attached to the pinnae of the ear. The end point following increasing temperature in the ear clip is taken as an ear flick or head shake (Nolan et al., 1985). The advantage of a thermal stimulus is that it is a natural pain stimulus and is easy to control. It's disadvantages are the risk of tissue damage, limitations in regards to the frequency with which measurements can be made and changes to skin temperature from drug effects or other manipulations causing spurious alterations of pain thresholds.

Chemical The subcutaneous or intra-peritoneal application of chemicals such as phenylquinone, brewer's yeast or bradykinin injected to produce writhing or vocalization suffer from slow onset and offset of stimuli. The frequency of measurements is also limited.

Mechanical Mechanical stimulus is probably the oldest of the pain-producing stimuli used in experimental animal work, and involves applying pressure to the skin or visceral organs to stimulate mechanoreceptors until a nociceptive response is observed. The amount of force required to elicit this response is recorded as the nociceptive end-point. A method has been developed for use in sheep (Nolan et al., 1987a) where application of a blunt pin to the fore-leg is used to elicit a leg lift. Whilst mechanical stimulus is natural and easily applied, it suffers from some limitations in that it is difficult to elicit pain repeatedly or frequently without producing receptor damage (Lineberry, 1981).

Electrical Electrical stimuli has been used to elicit pain in studies through electrodes on the skin, in sensory ganglia or dental pulp. The main advantages of electrical stimuli are that they are easily controlled despite fluctuations in resistance. They are easily applied and measured and high levels of pain can be induced with no apparent tissue damage. The rapid onset and offset also means that frequent measurements can be made. A nociceptive method using a ramped electrical stimulus in sheep has been developed by Ludbrook et al. (1995) for use in sheep. It has been used to define anti-nociceptive profiles for both anaesthetics (Ludbrook et al., 1996) and analgesics (Grant et al., 1996). This algesimetry method provides a highly sensitive and repeatable index of analgesia, the stimulus is brief and causes no damage to the testing area. Examination of baseline stability has shown that the threshold current required to produce a leg withdrawal response does not change over time. The main disadvantages to electrical stimulus are that it is not a natural stimulus and care needs to be taken not to activate other peripheral afferent systems.

1.2.3.2 Response types

Algesimetry techniques rely either on reflexive responses (such as tail flick and hot-plate methods) or learned behavioural responses that require a cognitive response by the animal to terminate the stimulus (such as those seen in ramped stimulus methods). The reflexive or unlearned response methods are widely used because of their convenience and reliability and can rapidly screen drugs as they require no special training of the subject. However, the responses seen are purely reflexive originating from the spinal cord. As such they give no indication of pain perception from higher centres, such reflexes can appear normal even in the spinally transected animal (Lineberry, 1981).

Learned responses where the subject initiates the cessation of the noxious stimulus are generally considered superior as they require a higher level of processing of the noxious stimulus. The disadvantages of these methods are that if simple escape tendencies are used to measure pain tolerance, the subject may halt the stimulus at the level of pain detection rather than pain tolerance. Stereotypical behaviours that are unrelated to the painfulness of the stimulus can also be exhibited in some animals, which may result in behaviours such as 'freezing' in response to obviously painful stimuli (Lineberry, 1981).

1.2.4 Behavioural indicators of pain

The use of behavioural indicators to assess the degree of pain in lambs following painful husbandry procedures is a relatively new technique. The measured behaviour types can be either spontaneously occurring behaviours in which the researcher is merely an observer recording the various activities, or behaviours exhibited by the animal as a result of the researcher interacting in some way with the animal. However, the range of expressive behaviours in sheep is more limited than those of many other species. This is probably due to the defenceless nature of sheep in relation to predators. Obvious manifestations of distress or weakness within a flock environment would make it a target to predators such as foxes or wolves. Nevertheless, simple behavioural indicators of pain were first shown to correlate with changes in the cortisol response to tail docking and castration in lambs (Mellor and Murray, 1989b). Since then further behavioural parameters have been added to the range of recorded indices (Molony and Kent, 1997; Thornton and Waterman-Pearson, 1999). This has provided a level of sophistication to the technique such that they offer an even more accurate discrimination between the severity of pain types than plasma cortisol levels (Molony and Kent, 1997). Behavioural indicators offer a method of assessing not only the severity of a painful treatment in sheep, but can also be used to measure the effectiveness any analgesic treatment for ameliorating painful behaviours.

1.2.4.1 Active pain behaviours

Active pain behaviour (APBs) can be of two types. The first is behaviour or activity that is only seen in response to discomfort or stress. The second is APBs that may be described as normal behaviours and can be exhibited even during non-stressful periods. When these behaviours are carried out to such a degree or repetition as to impair the normal functioning of the animal they act as a behavioural indicator of stress.

Active pain behaviours in sheep include restlessness, tail wagging, teat seeking, vocalization, foot stamping, rolling and licking/biting of the wound site (Mellor and Murray, 1989b; Kent et al., 1993). The measurement of these behaviours is a simple count of the number individual occurrences of each event over a given time period. From this an APB vs time profile can be compared against the incidence of similar behaviours in control animals.

1.2.4.2 Postural indicators of pain

Postural positions can also act as cues to discomfort or stress and have been used in determining the pain associated with husbandry procedures such as castration and tail docking (Molony et al., 1993; Kent et al., 1995). The postural indicators of pain are broadly divided into the two obvious categories based upon whether the sheep is recumbent or upright. Further classification within each group ranks the level of activity or degree of abnormality displayed (Graham et al., 1997). Thus, the range of postures for a standing sheep goes from apparent normal standing/walking to grossly abnormal standing/walking or complete immobilization. Likewise, the recumbent positions range from normal lying with legs tucked beneath the sheep to lateral lying with all legs extended, which is rarely observed during normal behaviour.

1.2.4.3 Aversion behaviours

A technique for determining an animals perception of the painfulness of a treatment is by measuring it's subsequent aversion to the same procedure. The basic procedure involved in aversion learning is to place a sheep in a race and measure the time it takes to run down the race and how much time was needed to push it if it resisted. At the end of the race the sheep receives some form of treatment. The sheep is then returned to the beginning of the race allowed to run through again and the treatment repeated. This pattern is repeated approximately 7 times. From this an objective indicator of the reluctance of the sheep to undergo a given treatment can be expressed in seconds of 'pushing time'. These techniques are sensitive enough to discriminate between the severity of treatments showing shearing to be more aversive than physical restraint (Rushen, 1996) and that electroimmobilisation (EIM) is more aversive than physical restraint (Rushen, 1986a).

An adaptation of this aversion learning technique is to allow sheep to choose between two treatments. For example, sheep are forced to travel down a Y-shaped race and, dependent upon which arm they travelled down, received either partial shearing or EIM. Sheep were given 2 forced choices initially being forced down one arm of the race then the other to receive treatments. Sheep are then given 12 free choices, receiving treatments based upon which arm of the race they choose to enter. Using such methods it appeared that sheep found EIM more aversive than shearing, as the preference for entering the shearing arm of the race becomes greater with subsequent repeats (Rushen and Congdon, 1986).

The limitations of aversion learning techniques are that they are dependent upon the learning ability of the sheep. For this reason, multiple treatments are required before it is possible to distinguish between control and stressful treatments. For example, with push times the aversiveness to the treatment appears to increase up to about the 5th treatment, then some form of adaptation occurs and the aversiveness to the treatment reduces (Rushen, 1986a). It is unknown whether this is an actual reduction in the aversiveness of the treatment or just a reduction in the novelty of the experience. Probably the most limiting factor in the use of these responses is that they can only be used where repeat treatments are possible, this obviously rules out husbandry procedures such as castration and tail docking that can only be performed once.

1.2.4.4 Evoked pain responses

Evoked pain responses are measures of pain in which the animal is disturbed during its normal behaviour and its response to this interaction is assessed. Two measures of evoked response have been used to assess the efficacy of analgesic treatments in lambs.

Unresponsive behaviours - Certain types of pain have a depressive effect on the alertness and activity of an animal (Morton and Griffiths, 1985). Unresponsive behaviour can be assessed by the observer interacting with the animal and recording the response to pen entry, capture and handling by the observer. These are scored on a sliding scale system ranging from 'totally responsive' to 'totally unresponsive'. Although unresponsive behaviours can be normal lamb behaviours and may merely be a manifestation of drowsiness, the incidence of these behaviours appeared to be markedly higher in lambs following painful castration procedures (Thornton and Waterman-Pearson, 1999).

Hypersensitivity - The hypersensitivity of an area that has undergone a painful treatment can be used to provide information on the animals perception of the pain and the amelioration of this pain due to analgesic interaction. In lambs undergoing a variety of castration methods, scrotal pain was assessed by recording the lambs response to palpation of the neck of the scrotum following castration. Responses ranging from,“no response” to “severe bucking” were scored on a sliding scale. The behavioural response to this palpation was able to differentiate between the ability of different anaesthetic regimens to provide pain relief following castration in lambs (Thornton and Waterman-Pearson, 1999).

One problem with the use of evoked responses, is that constant interaction alters the normal behaviour of the lambs, affecting the validity of active pain behaviours, postural and locomotory data. Measures of hypersensitivity are also difficult following some husbandry procedures. For instance, uniform degrees of palpation around the wound site following Mulesing would be difficult and may also slow healing.

1.2.5 Summary

The behavioural indicators are useful for determining the painfulness of a husbandry procedure. They offer an ethical, non-invasive method of measuring not only the pain following painful husbandry procedures in lambs, but also the ability of an analgesic agents to reduce that pain and suffering. Care must be taken to provide the right environment in which to record these behaviours as many other factors that are not pain related can influence behaviour. This is particularly so for evoked pain responses and aversion behaviours. Due to the limited range of behavioural indicators available for measurement, recording as many relevant individual behaviours as possible provides the most powerful tool for behavioural analysis.

1.3 Analgesic drugs used in sheep

1.3.1 Local anaesthetics

Local anaesthetics work by inhibiting the conduction of nerve impulses. At effective concentrations, local anesthetics block the transmission of autonomic, somatic sensory and somatic motor impulses. Their action is regional, dependent upon the method of administration, the most common method being local infiltration via subcutaneous injection. Spinal anaesthesia using local anaesthetic is a method of achieving pain relief in greater regions of the body and can affect the entire caudal half of the body, but it is rarely employed in general veterinary practice due to the technical difficulty.

Two local anaesthetics have been examined for their effectiveness in reducing pain following tail docking and castration in sheep.

1.3.1.1 Lignocaine

Lignocaine (or *Lidocaine) Hydrochloride* was first synthesized in 1944 and is probably the most widely used local anesthetic in clinical and veterinary practice. The absorption and elimination of infiltrated lignocaine is rapid with an effective duration of analgesia of about 90 minutes (Steffey and Booth, 1995).

Subcutaneous and intramuscular injection of 2% lignocaine solution into the surrounding tissue prior to tail docking and castration by application of tight rubber rings has been shown to reduce both the hormonal and behavioural indicators of stress in lambs (Kent et al., 1998; Wood et al., 1991). Intrathecal administration of lignocaine (0.6–1 mg/kg) provided effective analgesia in 8 month old rams during surgical castration, dependent upon the accuracy of the placement of needle during injection (Scott et al., 1996). However, subcutaneous and intra-testicular injection of 2% lignocaine provided no long term benefit for surgical castration as measured by a reduction in scrotal pain (Thornton and Waterman-Pearson, 1999).

1.3.1.2 Bupivicaine

Bupivicaine Hydrochloride was first synthesized in 1963, and is a long-acting local anaesthetic with a potency about 4 times that of lignocaine. In examining reductions in pain following castration in lambs, Molony et al. (1997) found that infiltration of 0.25% bupivicaine failed to reduce the hormonal and behavioural indicators of pain. Two factors may have been responsible for this:

1. An insufficient period between injection and castration (1–2 min) to allow for effective infiltration.
2. The site of injection was the scrotum, which was subsequently rendered ischaemic by rubber ring or crushing, thus preventing further perfusion and drug delivery.

When administered distal to the point of rubber ring, the application of bupivicaine was quite effective in reducing some of the abnormal postures indicative of severe pain (Graham et al., 1997).

1.3.1.3 Local anaesthetics summary

Local anaesthetics can be effective at reducing stress indicators following some painful husbandry procedures. There have also been developments using high pressure needleless injection that could make application simple for unskilled users (Kent et al., 1998). However, at this time there are flaws that appear to reduce its effectiveness, with limitations on the maximum volume being injected (0.2ml) and difficulties in penetrating woolled areas of the skin.

The shortcomings of infiltration with local anaesthetics are that their efficacy is confined to region around the site of injection. Some husbandry procedures, such as Mulesing, would require multiple injections in order to provide anaesthesia to the relatively large tissue areas concerned. Therefore, a lamb could theoretically require multiple injections to the ear, tail, rump and testes before commencement of husbandry procedures. Also, because these husbandry methods employ either ischaemia or complete removal of tissue areas there is the risk of either blocking the perfusion of the administration site or removing the tissue areas containing the anaesthetic intended to provide pain relief. Whilst spinal administration techniques can provide larger regions of anaesthesia, the need for strict asepsis and technical difficulties in achieving accurate administration make these methods unsuitable in general veterinary or farm practice.

1.3.2 Non-steroidal anti-inflammatories

There are many drugs that are classified under the broad heading of nonsteroidal anti-inflammatory drugs (NSAIDS), with the only requirement being the ability to reduce inflammation and that they are not steroidal. Whilst not primary analgesics, they do have the ability to reduce pain by reducing inflammation and have proven effective for many forms of ischaemic and visceral types of pain. The earliest of the NSAIDS, aspirin, was an important part of early herbal medicine and many of the NSAIDS are described in terms such as “aspirin-like”.

1.3.2.1 Diclofenac

Diclofenac is an anti-inflammatory, anti-pyretic that inhibits prostaglandin synthesis with a potency approximately 5 times that of aspirin. The efficacy of diclofenac appears variable in reducing pain behaviours following painful husbandry procedures. The administration of 1.5 mg/kg of diclofenac prior to castration by crushing of the spermatic cord using the burdizzo reduced plasma cortisol levels and some pain related behaviours when compared to unmedicated lambs (Molony et al., 1997). However, the same drug regimen confoundingly increased the incidence of abnormal behaviours in lambs tail docked using tight rubber rings (Graham et al., 1997).

1.3.2.2 Flunixin meglumine

An anti-inflammatory, anti-pyretic agent with a potency approximately 20 times that of aspirin, flunixin meglumine is used to treat musculo-skeletal disorders and colic pain in many species. Sheep suffering chronic footrot display elevated nociceptive thresholds to a mechanical stimulus, due to endogenous opioid response, an indicator of stress. Administration of flunixin meglumine (1 and 2 mg/kg IV) showed no benefit acutely (up to 6 hrs) but thresholds were reduced to control levels over 3 days, indicating the pain associated with footrot had been reduced (Welsh and Nolan, 1995). No changes in anti-nociception in response to a painful electrical stimulus were observed over a 60 minute period following the administration of flunixin meglumine 2mg/kg IM to sheep (Grant et al., 1996).

1.3.2.3 NSAIDS summary

The NSAIDs have shown some ability to reduce the indicators of pain resulting from footrot or crushing of the spermatic cord. However, there is no evidence or mechanistic basis to suggest they are suitable for treating the pain resulting from surgery that is not associated with inflammation.

1.3.3 Opioids

Opium has been used in human medicine since the dawn of history, and the opioids are the analgesic agent traditionally used for treating moderate to severe pain in many species. Thus far, four distinct types of opioid receptors have been identified and the different opioid drugs exert slightly different effects dependent upon their affinity for various opioid receptor types:

mu (μ) There are two receptor subtypes, $u_1$appears to be responsible for analgesic effects while the u_2 is responsible for respiratory depression.

delta (δ) Show greatest sensitivity for endogenous opioids.

kappa (κ) Produces anti-nociception, sedation and dysphoria

sigma (σ) Does not mediate analgesic effects

Most of the opioids we define as analgesics exert the majority of their effects upon the μ receptor but often, especially at higher doses, the dysphoria and agitation produced by κ receptor effects become apparent. There have been a number of studies examining the analgesic efficacy of different opioids in sheep.

1.3.3.1 Morphine

Morphine Sulphate - was the first of the opium plant alkaloids to be synthesized in 1805. The μ receptor was so named because of high binding affinity morphine has for that particular receptor. Epidural morphine (5 mg), produced relatively ineffective anti-nociception to a painful electrical stimulus in the sheep, with only 2 data points (a 10 minute period) where the avoidance threshold was above control values (Eisenach et al., 1987).

1.3.3.2 Methadone

Methadone Hydrochloride - was first synthesized in Germany in 1941 in the search for a synthetic replacement for morphine. It has a high binding affinity for the μ receptor with a potency 3 times that of morphine. Algesimetry studies using an electrical stimulus showed no changes in anti-nociception in doses up to 1 mg/kg IM (Grant et al., 1996).

1.3.3.3 Pethidine

Pethidine Hydrochloride - IV administration of pethidine (2.5 and 5 mg/kg) causes brief (3–10 min) increases in anti-nociception to painful thermal and mechanical stimulus (Nolan et al., 1985, 1987a). Both doses also caused agitation.

1.3.3.4 Buprenorphine

Buprenorphine Hydrochloride - is a partial μ agonist with a slow onset and long duration of action with 10 times the potency of morphine. Algesimetry studies using a painful thermal stimulus showed increases in anti-nociception for approximately 3 hours at doses of 6 μg/kg IV. This dose also produced agitation and restlessness that confounded interpretation of behavioural end-points (Nolan et al., 1985, 1987b,a). No changes in anti-nociception were seen in response to similar testing regimens using mechanical stimulus (Nolan et al., 1987b). IM administration of 5 μg/kg of buprenorphine produced no restlessness or agitation but also no changes in anti-nociception to a painful electrical stimulus (Grant et al., 1996).

1.3.3.5 Butorphanol

Butorphanol Tartrate - is another synthetic morphine derivative. A partial agonist-antagonist it acts primarily as an antagonist at the μ receptor and an agonist at the κ receptor. As an agonist it is 4–7 times more potent than morphine. Sheep administered butorphanol at doses of 0.05, 0.1, 0.2 mg/kg IV showed increases in anti-nociception to a painful thermal stimulus for 60–160 minutes at all doses. The two higher doses also produced pronounced dysphoric behaviours in the sheep such as agitation and chewing. Algesimetry testing using a mechanical stimulus at the same doses showed no changes in anti-nociception (Waterman et al., 1991).

1.3.3.6 Fentanyl

Fentanyl Citrate - a full agonist active at μ, κ and δ receptors. It is more lipid soluble and 30 times more potent than morphine producing a more rapid onset and shorter duration of action.

The literature regarding the efficacy of fentanyl in the sheep during algesimetry testing is difficult to interpret. 5 μg/kg IV of fentanyl has been shown to provide approximately 35 minutes of increased anti-nociception (Nolan et al., 1987a), or else no change (Kyles et al., 1991, 1993b). Co-administration of the neuroleptic, droperidol, causes either a synergistic increase in anti-nociception (Kyles et al., 1991) and agitation (Kyles et al., 1993b) or reduces fentanyl induced dysphoria and causes sedation (Livingston et al., 1991).

1.3.3.7 Opioids summary

As may be expected, opioids constitute a large portion of the algesimetry testing performed in sheep. This no doubt stems from the widespread clinical use of opioids and their success in producing analgesia in many other species.

However, a number of strong arguments against the routine use of opioids as an analgesic in sheep can be given:

- they appear to lack analgesic action in sheep (Nolan et al., 1987b; Grant et al., 1996).
- produce unwanted behavioural effects such as agitation and dysphoria (Westhues and Fritsch, 1965; Livingston et al., 1991; Ludbrook et al., 1995).

Many of the opioid findings appear conflicting, a given drug and dosage may be termed an analgesic in one study and described as ineffective or a dysphoric in another. Even in the hands of the same authors variable responses in anti-nociceptive testing can be seen. The reasons for this may be due to differences in administration route that can influence action and bioavailability (Baggot, 1992) or differences in algesimetry testing methods that can stimulate different afferent nerve fibres producing different pain types. This may be the reason for differences seen in the anti-nociceptive profiles between mechanical and electrical stimulus following identical opioid administration regimens.

Importantly, no opioid has demonstrated obvious anti-nociceptive effects in sheep without the confounding effects of agitation or dysphoria, that would make interpretation of the nociceptive responses difficult. A purposeful ear flick or leg lift in response to painful stimulus could easily be obscured in the increase by locomotor activity, chewing, bleating etc.

Studies of opioids in the sheep pose unique questions. Opioid receptors are obviously present in the sheep but as discussed above their function in providing analgesia is unknown. Even the role of endogenous opioids in producing analgesia in sheep remains unclear, with studies of tail docking showing that the reduction in pain from endogenous opioid release is only small (Wood et al., 1991). It seems that despite the 'wealth' of algesimetry data regarding sheep and opioids, long held recommendations and anecdotal evidence regarding the unsuitability of opioids for pain control in sheep can be maintained (Westhues and Fritsch, 1965).

1.3.4 Alpha-2 agonists

Based upon their pharmacological properties, the adrenergic receptors were subdivided into α and β subtypes in 1948. The α receptors were later further divided into α_1 and α_2, subtypes based upon their location and function. The analgesic and sedative effects caused by α_2 adrenoceptor agonists are similar to those seen from the μ opioid agonists. Although they act upon different neuronal receptors, both agonists cause their effects by activating cell membrane G-proteins to open potassium channels and hence are functionally similar (Ley et al., 1991). However, α_2 agonists can also produce hypotension and respiratory and cardiac depression (Klide et al., 1975; Wagner et al., 1991; Celly et al., 1999). The analgesic effects of the α_2 agonists appear to be mediated spinally via receptors on the dorsal horn (Kyles et al., 1993a), whilst receptors on blood vessel walls and cardiac tissue are assumed to be responsible for the cardiovascular effects (Maze and Tranquilli, 1991).

1.3.4.1 Xylazine

Xylazine hydrochloride - was synthesized in Germany in 1962 and was the first α_2 adrenoceptor agonist to be used by veterinarians. It is now widely used as a sedative and analgesic in many species. Ruminants, such as sheep, appear to be particularly sensitive to xylazine and the therapeutic dose range is small (Westhues and Fritsch, 1965), higher doses producing sedation and adverse cardiopulmonary effects (Celly et al., 1997). There is also evidence of a possible variation in dosing requirements between different breeds of sheep (Ley et al., 1990).

The analgesic effects of xylazine have been displayed in sheep under a variety of different administration routes and algesimetry testing methods. Low dose xylazine (50 μg/kg) has displayed significant anti-nociceptive effects to thermal (Nolan et al., 1985, 1987a), mechanical (Nolan et al., 1987a; Ley et al., 1990) and electrical (Grant et al., 1996) algesimetry testing methods. Significant increases in anti-nociception have been observed following intravenous (Nolan et al., 1985, 1987a; Ley et al., 1990), intramuscular (Grant et al., 1996) and intrathecal (Waterman et al., 1988) administration of xylazine. All these studies showed significant increases in anti-nociception for at least 60 minutes, and the magnitude of these changes was dose dependent (Waterman et al., 1988; Grant et al., 1996).

1.3.4.2 Other alpha-2 agonists

Clonidine - a selective α_2 agonist has been used in human medicine as an anti-hypertensive for many years. Spinal administration of clonidine in sheep has demonstrated anti-nociception to electrical (Eisenach et al., 1987) and mechanical (Waterman et al., 1988) stimuli. The mechanical stimulus testing showed a potency approximately twice that of xylazine.

Detomidine hydrochloride -One of the newer α_2 agonists, it is more potent and has a greater specificity for α_2 receptors than xylazine. IV doses from 2–7 μg/kg showed dose dependent increases in anti-nociception to a mechanical stimulus. Sedative effects were also noted at these doses (Muge et al., 1994).

Dexmedetomidine hydrochloride - Another of the new α_2 agonists. 100 μg of intrathecal dexmedetomidine produced 90 minutes of increased anti-nociception to a mechanical stimulus, and significant hypotension (Eisenach et al., 1994).

1.3.4.3 Alpha-2 agonists summary

Of all the analgesics, the α_2 agonists demonstrate the most consistent analgesic effects in sheep in response to a variety of testing and administration methods. The efficacy of the real world application of xylazine to treat pain following painful surgical procedures in sheep remains unknown. Other challenges to its routine use are the incidence of detrimental cardiovascular and respiratory side effects. As α_2 agonists mediate their analgesic effects via the spinal cord, their cardiovascular effects via receptors located on the arterial and venous vasculature, and their respiratory effects via circulating platelets (Eisenach, 1988). This means that it may possible to achieve pure analgesia and reduce deleterious respiratory or cardiovascular effects by using spinal administration methods (Eisenach et al., 1987; Castro and Eisenach, 1989). All deleterious cardiovascular and respiratory effects have been noted following intravenous administration (Waterman et al., 1987; Celly et al., 1997). Intramuscular administration may offer a much technically simpler method to reduce these effects by minimising direct stimulation of the peripheral α_2 receptors in the vasculature or as a result of altered drug absorption profile.

1.3.5 Summary

The four classes of drugs reviewed act upon different receptors and provide different forms of pain control. The choice of a suitable analgesic in the 'field' environment can be complicated by different husbandry procedures stimulating different afferent nerve fibres and receptors. Large wool producers in Australia tend to favour cost-effective husbandry procedures and use surgical methods of castration and tail docking, whilst farmers in the UK have smaller flocks and employ rubber ring tailing and castration methods and do not perform the mulesing operation. Thus the choice of analgesic needs to be based upon the husbandry methods employed. The research examining pain during husbandry procedures reviewed above often reflects the local methods employed.

Of the four groups of analgesics reviewed, the α_2 agonists show the most promise for providing simple, effective pain control for sheep provided issues related to cardiovascular and respiratory depression can be resolved. They have been shown to be efficacious under a variety of testing and administration paradigms. Local anaesthetic agents appear effective at providing site specific pain relief for castration and tail docking but are incapable of treating less localised pain sources. It is possible that NSAID's may reduce the painfulness of ischaemic tailing and castration methods but there is no evidence of their ability to treat pain from surgical procedures. Any interpretation of analgesic effects seen following opioid administration were generally confounded by dysphoria and agitation.

1.4 Summary and aims of research

This review has shown that many commonly used analgesics are unsuitable for providing simple methods of pain relief to sheep following surgical procedures. The α_2 agonists undoubtedly produce dose dependent anti-nociception, but as with many other analgesics their ability to reduce surgical pain still remains untested. Xylazine, the most widely used and easily accessible of all the α_2 agonists appears as efficacious as the newer α_2 agents and is an obvious choice for providing routine analgesia in sheep.

However, some outstanding issues regarding its use in sheep and lambs need to be addressed. The cardiovascular and respiratory effects seen following intravenous administration of xylazine would prove detrimental for its routine use as an analgesic agent, however these effects may be reduced or abolished following intramuscular administration due to an altered absorption profile. Intramuscular administration also provides the simplest administration method requiring little or no specific technical expertise, unlike spinal or multiple injection site methods. There has been no algesimetry testing of any analgesics in lambs, as such it is not known whether lambs demonstrate the analgesic effects seen in adult sheep following xylazine administration. This should be determined before it can be used to provide pain relief for lambs undergoing painful husbandry procedures.

These issues can be addressed by measuring the cardiovascular effects of known analgesic doses of intramuscular xylazine in the sheep. Algesimetry methods can be used to compare the anti-nociceptive response of lambs to this dose, for comparison to similar data from adult sheep. Once the cardiovascular safety of this dose of xylazine is assured and differences in the therapeutic dose range between adult sheep and lambs are known, administration regimens for dealing with common painful procedures in sheep such as husbandry procedures and the management of post-operative pain can be developed.

The specific aims of this thesis were:

1. To examine the differences in analgesic efficacy between intramuscularly, intravenously and subcutaneously administered xylazine in sheep.
2. To determine the cardiovascular safety of low dose intramuscular xylazine administration in sheep.
3. To develop an analgesic protocol to provide predictable, effective, steady-state analgesia in sheep based upon the administration of intramuscular and intravenous xylazine.
4. To examine the anti-nociceptive effects of low dose xylazine in lambs, and to determine if any variation in effect exists between lambs and adult sheep.
5. To examine if the use of intramuscular xylazine can provide a reduction in pain for lambs undergoing painful husbandry procedures.

Chapter 2

General methods and materials.

2.1 Ethical approval

All studies in this thesis were approved by the Animal Ethics Committees of The University of Adelaide and The Institute of Medical and Veterinary Sciences, Adelaide, South Australia.

2.1.1 Ethical considerations

Studies requiring the chronic catheterisation of sheep were performed using sheep previously prepared for other unrelated pharmacokinetic studies. This maximized the utility of each experimental preparation and reduced the total number of sheep used within the laboratory.

The acute painful stimulus used in algesimetry studies in this thesis was brief and caused no damage to the testing area. Trials of the device on the author showed that the point at which unmedicated sheep terminated the current could be equated to a mildly uncomfortable experience rather than overt pain (Ludbrook et al., 1995). In the author's experience, sheep that find the algesimetry testing regimen unpleasant respond with behavioural displays (e.g. foot stamping) that make it impossible to acquire useful readings. These sheep therefore exclude themselves from participating in algesimetry studies.

The husbandry procedures in lambs described in this thesis were performed to benefit productivity and lamb health and were part of the normal husbandry practices performed on the farm where studies took place. No additional husbandry procedures were performed for the benefit of this thesis, in the context of the farms normal operation nor in terms of general wool farming husbandry practices in Australia.

2.2 Animal selection and handling

All sheep (Ovis Aries) and lambs used in these studies were Merino breed, purpose bred for experimentation at Windarra farm of the Institute of Medical and Veterinary Sciences. Selection of sheep from a single flock with common blood lines reduced any variation due to genetic differences.

2.2.1 Adult sheep

Adult sheep used in algesimetry and cardiovascular studies were between 1.5 and 3 years of age and of a similar weight. Sheep were housed in the animal house facility of the Faculty of Health Sciences at the University of Adelaide, and cared for according to the guidelines for animal care of the National Health and Medical Research Council of Australia (National Health and Medical Research Council, 1995). Sheep were kept within mobile metabolic crates with free access to feed and water in a climate controlled room with an automatically timed spring lighting cycle. Sheep were always housed at least in pairs to avoid isolation stress. Faeces and urine output, and food and water consumption, were monitored daily. Sheep were housed in this environment for at least 5 days before surgery or studies commenced to allow them to adapt to their surroundings.

2.2.2 Lambs

Lambs used in behavioural studies were the offspring of sheep from the Merino flock at Windarra Farm. All lambs were part of a normal flock structure, and other than on the day of behavioural observations, remained in large paddocks with the flock and their mothers. Care was taken not to perform studies or handle sheep during periods of adverse weather that may have caused stress to the sheep. All handling and 'rounding up' procedures were performed in the standard fashion by the farm manager and would not have presented a novel experience to sheep.

2.3 Instrumented sheep preparation

2.3.1 Anaesthesia

All surgical procedures involving catheterisation of sheep were performed under general anaesthesia. Sheep were induced within their crates by rapid injection of 1000 mg sodium thiopentone (Abbott Australasia, Kernell, NSW, Australia) into the left internal jugular vein. Sheep were then transferred to the surgical table, placed on their back and intubated using a cuffed endotracheal tube with the aid of a laryngoscope. Correct placement was confirmed by monitoring end-tidal carbon dioxode tension using an infra-red carbon dioxide analyser (Model OIR 7101, Nihon Kohden Corporation, Tokyo, Japan). Anaesthesia was maintained using 2% halothane (Zeneca, Cheshire, England) in oxygen delivered via a vaporiser and circle system. Normocapnia was maintained by artificial ventilation using a gas powered ventilator and monitoring of end-tidal carbon dioxide tensions

2.3.2 Surgery

Strict aseptic techniques were used to implant catheters according to the general method of Runciman et al. (1984a). In brief, the neck was shorn of wool and the right carotid artery and jugular vein were exposed via a longitudinal skin incision. All catheters (Multi purpose A1 catheter, Cordis Corporation, Miami, FL, USA or 7F polyethlyene, Cook Australia, Eight Mile Plains, Qld, Aus) were inserted using Seldinger technique and correct positioning confirmed using fluoroscopy (Siremobil 2, Siemens, Germany) and injection of radio opaque contrast medium (Angiografin, Schering AG, Germany). For arterial blood sampling and measurement of arterial blood pressure, two 7F catheters were placed in the carotid artery with their tips positioned just above the brachiocephalic trunk. A 7F catheter was inserted in the jugular vein for drug administration with its tip positioned in the right atrium. A thermodilution catheter (Biosensors, Singapore) for the measurement of cardiac output was also placed in the jugular vein with its tip located in the pulmonary artery. All catheters were fastened to a plastic plate attached to the strap muscles of the neck and exteriorised.

Extension lines terminated with a three way stopcock (Abbott Laboratories, North Chicago, IL, USA) were connected to the catheters and lines kept patent by filling the volume of line and catheter with 50 IU/ml sodium heparin (David Bull Laboratories, Melbourne, Australia) and capping.

All sheep were able to stand and were eating within 3–4 hours of surgery. Post-operative analgesia was provided with xyalzine 2.5mg IM (Troy Laboratories, Smithfield, NSW, Australia).

2.3.3 Maintenance of instrumented sheep

All heparin locked catheters were flushed at least every three days to ensure their patency. If a catheter became blocked repeated flushing with sterilised heparinised 0.9% saline was attempted to either withdraw the clot or remove foreign material from the tip. If this failed, the catheter was filled with a stronger solution of sodium heparin (1000 IU/ml), capped and left for 24 hours. The exact volume of catheters plus extension lines was known and care was taken to inject as little heparin as possible systemically in the sheep. If these techniques all failed to recover the patency of the catheter and the sheep was no longer able to be used in studies, it was euthanased by intravenous administration of sodium pentobarbitone (Sigma Chemical Company, Castle Hill, NSW, Australia), in keeping with local legislation.

2.3.4 Laboratory studies

Sheep were allowed to recover from surgery for at least 1 week before experimental procedures commenced. On the experimental day the sheep were transported within their metaboilc crates to an experimental room at the University of Adelaide, Medical School Animal House facility. Sheep were placed in a sling within their crate that allowed them to partially weight bear but prevented them from lying down and influencing any haemodynamic measurements or preventing algesimetry readings.

2.4 Algesimetry studies

2.4.1 General methods for algesimetry studies

The algesimetry method was based based on a leg withdrawal response to a subcutaneous electrical stimulus produced by a modified transcutaneous nerve stimulator device. The analgesic profile of a drug was determined from the increase in current required to induce a learned leg withdrawal response (Ludbrook et al., 1995). Two 26G needles were placed subcutaneously in the anterior aspect of the lower third of the sheep's hind limb to act as electrodes between which the current could pass. The operator of the device was positioned alongside the sheep where any movement of the hindlimb was clearly visible (Figure 2.4.1). The nerve stimulator (Digistim 3, Neuro Technologies, Houston TX USA) was modified by a qualified biomedical engineer to deliver pulsed DC current to the needles increasing stepwise between 0 and a maximum of 50 mA over a period of approximately 5 seconds. The device automatically compensates for changes in skin resistance between the electrodes.

The operator depressed the hand-held cutout button and initiated the delivery of current. The end-point was taken as a deliberate withdrawal of the leg at which point the current was terminated by releasing the cutout button and the highest current level recorded. Readings were normally taken every 30 seconds. When end point current thresholds appeared stable for a period of at least 5 minutes, the readings were considered to be at a baseline and xylazine was administered.

This algesimetry method provides a highly sensitive and repeatable index of analgesia, the stimulus is brief and causes no damage to the testing area.

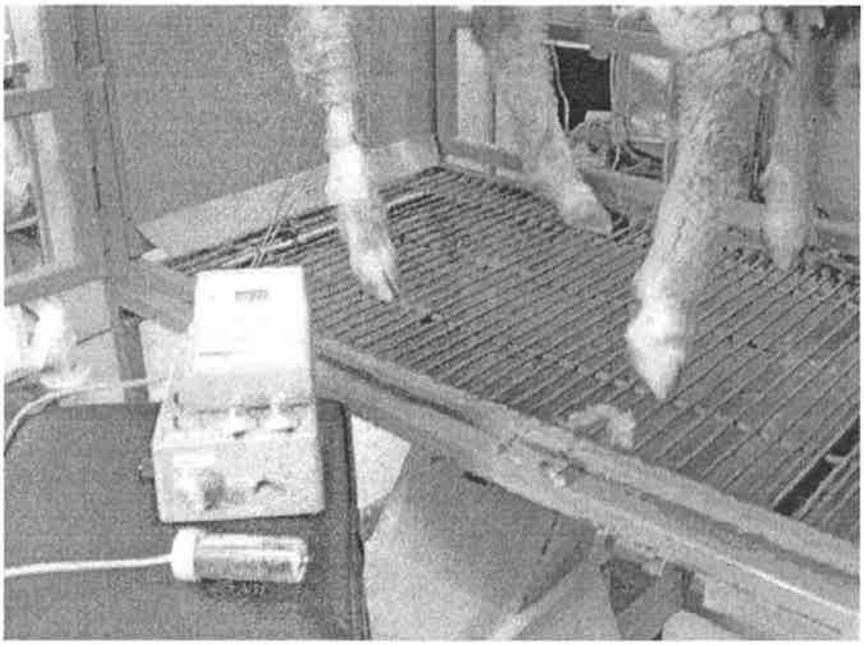


Figure 2.1: Algesimetry setup showing analgesiometer in foreground and wires leading to electrodes on lower hind leg of sheep. The sheep is supported by a sling within its metabolic crate.

Examination of baseline stability has shown that the threshold current required to produce a leg withdrawal response does not change over time (Ludbrook et al., 1995).

During the course of each study sheep were observed for signs of sedation such as increased salivation, the degree of ptosis and changes in alertness and response to external stimuli such as noise. At times of maximal drug effect (when threshold currents were at their highest values), sheep were given an innocuous non-painful stimulus such as gently touching the leg. If the sheep failed to withdraw its leg from this novel stumulus it was considered to be indicative of a depression of the central reflexes. From this, it could be determined if changes in end-point current thresholds were due to 'real' analgesic effects or due to the sheep's inability to respond as a result of inhibited locomotor activity.

2.4.2 Dosing in adult sheep

Classically in veterinary medicine dosing is expressed in terms of dose per kg, this is done to provide an easy 'rule of thumb' for calculating dosing requirements across a broad range of body weights. We have found that in adult sheep of the same blood line the variation in body weight is small and dependent mostly upon fleece length and rumen contents. Normalising doses for mass under such circumstances can contribute to variability of effect rather than reduce it. The determinants of kinetic processes actually scale better to lean body mass rather than total body weight, but measurement of lean body mass is difficult. With this knowledge, drug doses in adult sheep were not scaled for body weight. The nominal weight of the adult sheep used was 50 kg.

2.5 Behavioural studies

The general methods for the recording and measurement of lamb behaviours are described in Chapter 7.

2.6 General data handling and statistical analysis

Spreadsheets (Star Office 5.0, Star Division Corp.) were used extensively for data handling, storage and manipulation of data. Custom programs for data manipulation and entry were writtten by the author using the programming language Python (http://www.python.org) as indicated in subsequent chapters.

All statistical analyses were performed using the statistical programming language 'R' (Ihaka and Gentleman, 1996) or Statistica (Statistica for Windows

5.1, Statsoft Inc, Tulsa, USA). $P < 0.05$ was considered statistically significant.

Data were presented as mean ± SEM where it is intended to show the precision of the estimate of the population mean, and are shown as mean ± SD when it is intended to show the variability of the measurement.

Chapter 3

A comparison of the antinociceptive effects of xylazine via three different administration routes

3.1 Introduction

As concluded in chapter 1, the α_2 adrenoceptor agonist xylazine appears suitable for providing pain relief following surgical or painful husbandry procedures in sheep. Algesimetry testing methods can provide information on the depth and duration of analgesic effects in response to acute pain, and such methods have shown the substantial antinociceptive effects of xylazine following intravenous (Nolan et al., 1985; Ley et al., 1990; Nolan et al., 1987a) and intrathecal administration (Waterman et al., 1988; Kyles et al., 1993a). Similar increases in anti-nociception have been reported following intramuscular administration, but the duration of effect is not known (Grant et al., 1996).

The choice of administration routes can affect the efficacy of many drugs. While a dose given the intravenous route is 100% available to the systemic circulation, extravascular injection can reduce both the rate and extent of drug absorption. This can be due to factors such as the vascularity of the injection site and the degree of ionization and lipid solubility of the drug (Baggot, 1995). This effect can be either a reduction in the magnitude of peak analgesic effects, or a delay in the time course of analgesic effects. Indeed, the selection of administration route may provide a mechanism by which the analgesic profile can be better matched to the expected time-course of the pain experience.

The success of a pain relief regimen when incorporated into existing procedures is also dependent upon the ease of its application. Regimens that are technically difficult to employ will only be adopted by groups that have the skill to use them. Intravenous or spinal administration of analgesics can be appropriate methods of pain relief following surgical procedures, in an environment where veterinarians, researchers or animal carers are available to provide them. However, in normal farming environments such techniques are unsuitable due to the lack of available technical skill, and issues related to time efficiencies and cost effectiveness when dealing with large numbers of animals. Intramuscular and subcutaneous injection are simpler methods of administering analgesics, may be more easily employed in farming environments.

Before recommendations for pain control can be made, variations in the analgesic profile caused by different administration routes need to be assessed. The aim of this study was to examine differences in the anti-nociceptive effects of xylazine following intravenous, intramuscular and subcutaneous administration routes in sheep with respect to their potential for the management of pain from routine surgical and husbandry procedures.

3.2 Methods

3.2.1 Animal preparation

Adult Merino ewes between 1.5 and 3 years and approximately 50 kilograms were chronically instrumented as described in Section 2.3. Each sheep received xylazine via three different administration routes on different experimental days in random order. On the experimental day sheep were transported to the experimental room within the Faculty of Health Sciences animal house at the University of Adelaide, placed in a sling and prepared for algesimetry studies as described in Section 2.4.1 of the Methods chapter.

3.2.2 Study design

After a period of approximately 30 minutes to acclimatise to their surroundings, baseline algesimetry values were recorded and sheep were administered 2.5 mg of xylazine by one of the routes described below.

3.2.2.1 Intravenous administration

A 0.1 mg/ml solution of xylazine was prepared in a 50 ml syringe by diluting 3 mg of xylazine in 30 ml of 0.9% saline. Saline was chosen as the vehicle because intravenous infusions of hypotonic solutions, such as when some drugs are prepared in water, have been shown to produce cardiovascular perturbations in sheep (Huang et al., 1996). The syringe was then connected to the three-way stopcock terminating the jugular vein catheter via a minimum volume extension line. The extension line between the syringe and three-way stopcock was then primed with the infusion solution, and a syringe on the stopcock was filled with a volume equivalent to the deadspace of the catheter and extension line (approximately 2.1 mls).

After baseline values were recorded, the dead space between the stopcock and the animal was primed using the syringe at the stopcock, effectively priming the entire infusion line and catheter length. A programmable pump (Model 33, Harvard Apparatus Ltd, Kent, England) was then used to deliver a 2 minute infusion of the xylazine solution at 12.5 ml/min. Algesimetry readings were continued until end-point current thresholds had returned to baseline for at least 10 minutes.

3.2.2.2 Intramuscular administration

After baseline values had been recorded, 2.5 mg of xylazine prepared in 1 ml of 0.9% saline was injected into the rump of the contra-lateral leg to which the electrodes for algesimetry were attached. Algesimetry readings were continued until end-point current thresholds had returned to baseline for at least 10 minutes.

3.2.2.3 Subcutaneous administration

After baseline values had been recorded, 2.5 mg of xylazine prepared in 1 ml of 0.9% saline was injected under the wool free area of skin between the inside front leg and sternum. The skin was tented and great care taken to ensure correct needle placement at the time of injection. Algesimetry readings were continued until end-point current thresholds had returned to baseline for at least 10 minutes.

3.2.3 Data analysis

Algesimetry values were expressed as a percentage change from each sheeps' own baseline values. This was done to account for differences in the point at which sheep terminate the current by eliciting a leg lifting response. These differences can be due to the variation between each sheep in their ability to tolerate the painful stimuli, differences in skin conductivity between the electrodes and differences in the electrode needle placement on the foreleg. Although great care was taken to ensure that the position, depth and distance between the needles was consistent between sheep, small variations can cause differences in the current density ($mA \times cm^2$).

The total anti-nociceptive response was determined by measuring the area under the curve (AUC) between the time of injection and the 90 minute time point using the trapezoidal rule. The maximal analgesic response was calculated by averaging the maximum end-point current value of each sheep for the experimental period for each administration method.

3.2.3.1 Statistical analysis

To determine any statistically significant change in the antinociceptive response, the 95% Confidence Intervals (CI) for the difference between the means of the baseline data and time points following xylazine administration were calculated. If the upper and lower limits of the 95% CI did not include 0, there was assumed to be a significant difference from baseline at that time point. The two groups were assumed to have equal numbers of data points (Motulsky, 1995).

Paired t-tests were used to assess any difference in the maximum and total anti-nociceptive response (AUC) between different administration methods. A

P value of less than 0.05 was considered significant. Statistical analysis was performed using the data analysis language 'R' (Ihaka and Gentleman, 1996).

3.3 Results

Intravenous administration of xylazine demonstrated the fastest onset of all administration routes with the shortest time to peak algesimetry values (Table 3.1). However, this was also associated with the shortest period where algesimetry values were significantly above baseline (Figure 3.1) and 3 sheep showed signs of sedative effects with increases in the degree of ptosis and salivation. These changes were seen during the 30 minute period immediately following administration, but did not prevent the sheep from responding to non-painful stimuli such as touching the forelimb. No overt signs of sedation were observed during the intramuscular or subcutaneous studies.

Intramuscular administration of xylazine showed the greatest total antinociceptive response (AUC) and subcutaneous the least response (Figure 3.2), however these differences were not statistically significant. A comparison of the AUC of algesimetry values vs. time revealed that except for the first 15 minute period, intramuscular administration provided the largest anti-nociceptive response for each time period (Figure 3.3). The period for which algesimetry values were significantly above baseline was similar for both the intramuscular and subcutaneous administration methods (Figure 3.1).

3.4 Discussion

Algesimetry testing using a painful electrical stimulus showed all three administration routes resulted in substantial increases in anti-nociception. As

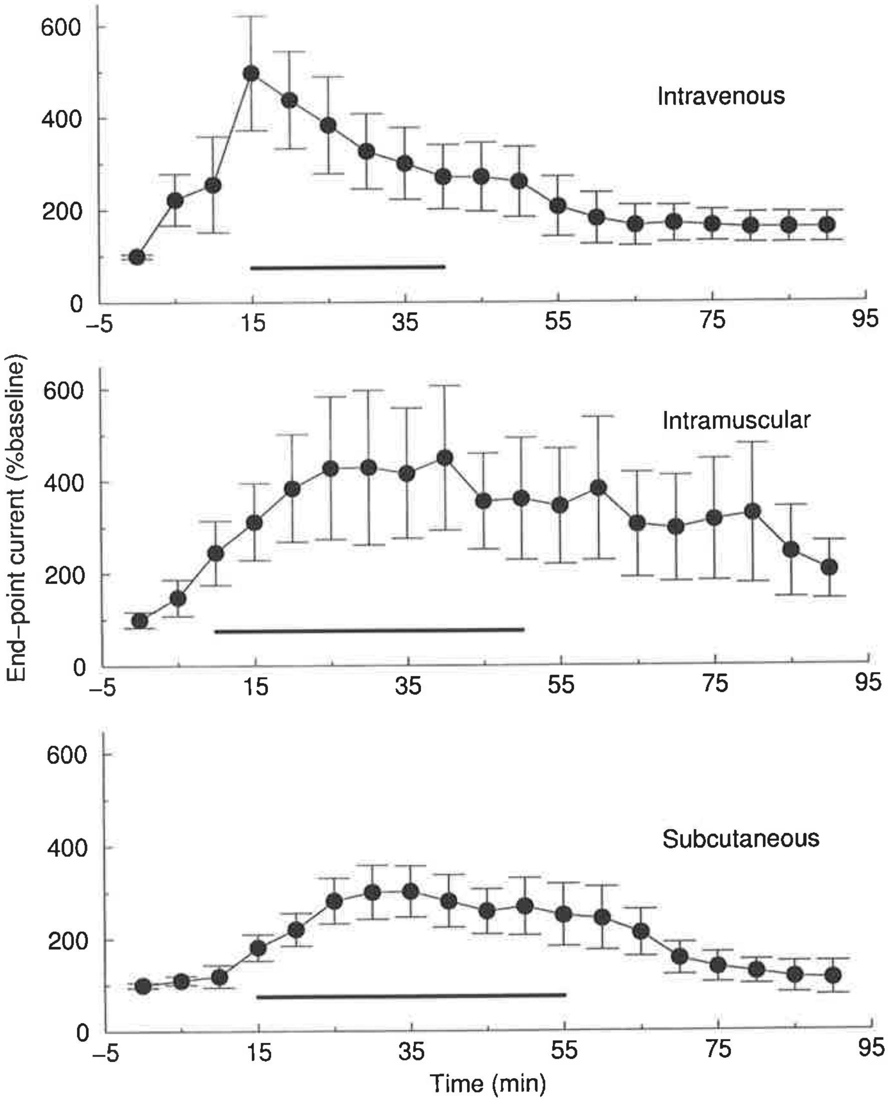


Figure 3.1: Anti-nociceptive effects of 2.5 mg xylazine following intravenous, intramuscular anwhered subcutaneous injection in 7 sheep. Thick bars indicates periods where values were different from baseline. Data are presented as mean ± SEM.

	Intravenous	Intramuscular	Subcutaneous
Baseline average (mA)	4.1 ±1.1	2.7 ± 0.6	4.5 ± 0 .9
Peak current threshold (mA)	27.1 ± 8.7	16.5 ± 6.6	16.5 ± 2.4
Time to peak current threshold (min)	13.8 ± 1.8^{Aa}	25.1 ± 3.9^{a}	29.9 ± 2.2^{A}

Table 3.1: Baseline and peak end-point current values following 3 different administration routes of 2.5 mg xylazine in 7 sheep. Data are presented as mean ± SEM. Superscipt in capitals indicates $P < 0.01$, lower case superscript $P < 0.05$.

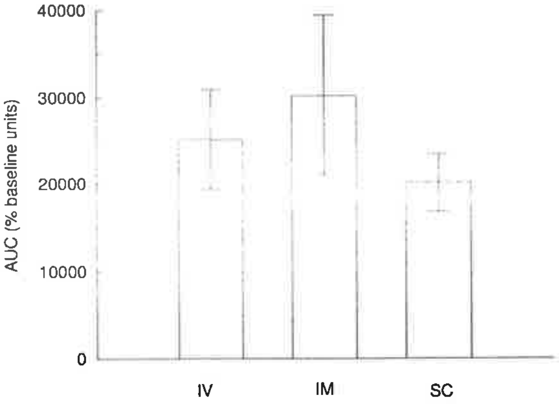


Figure 3.2: Total antinociceptive response (AUC) in 7 sheep following subcutaneous (SC), intramuscular (IM) or intravenous (IV) administration of 2.5 mg xylazine in 7 sheep. Data are presented as mean ± SEM.

expected for a route with no absorption delay, intravenous administration of xylazine was characterised by a rapid but relatively brief increase in antinociception. These findings are consistent with previous algesimetry studies using mechanical and thermal stimuli (Nolan et al., 1987a; Ley et al., 1990). The sedative effects of xylazine become more apparent with increasing dose (Maze and Tranquilli, 1991). The sedation observed in 3 sheep following IV administration was probably due to the rapid mixing of the drug with blood and the consequent high initial blood concentrations of xylazine anticipated for this route.

Intramuscular injection of xylazine resulted in the greatest total antinociceptive response, as measured by AUC, due to to the achievement of relatively rapid peak analgesia followed by slow drug wash out. Differences in the baseline algesimetry values between intramuscular and the other two administration groups (Table 3.1) were likely due to differences in skin conductivity between the electrodes, or differences in the electrode needle placement on the foreleg causing changes in the current density across the electrodes. A variation in behavioural response between sheep to the painful

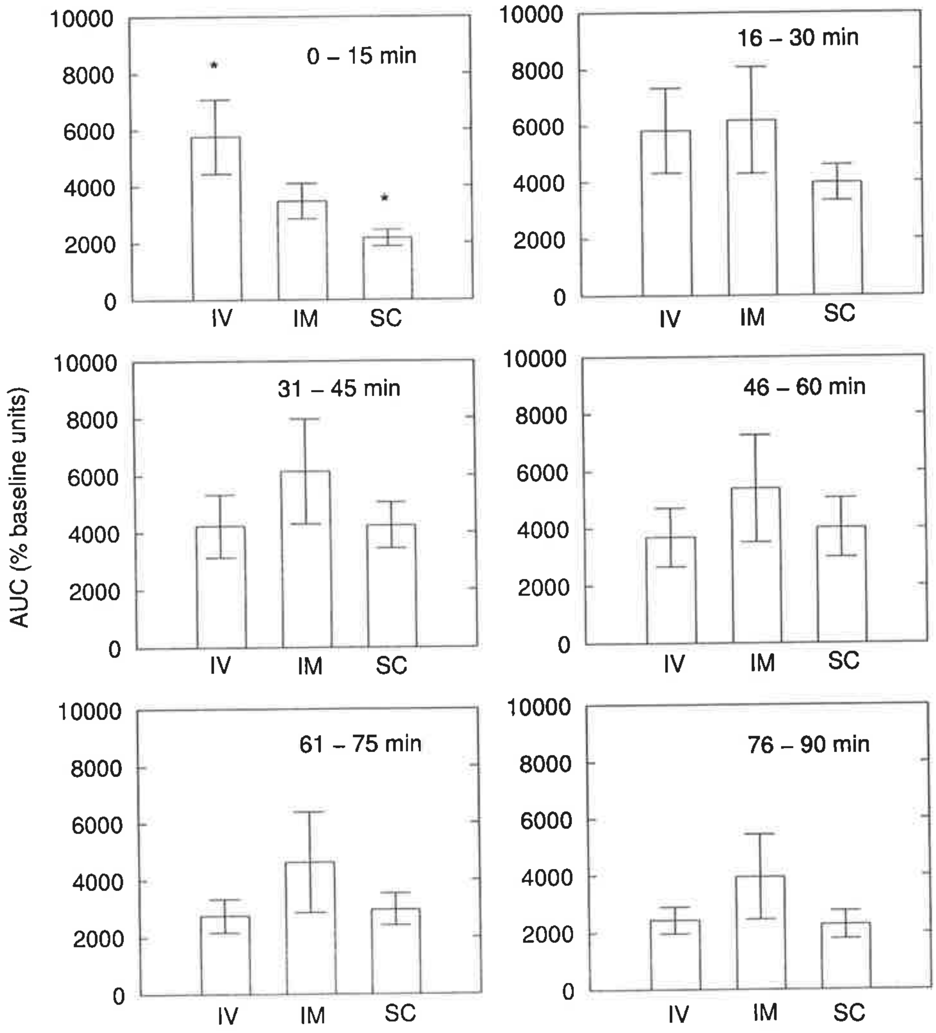


Figure 3.3: A comparison of the AUC of the anti-nociceptive response vs. time curves following administration of 2.5 mg xylazine either intravenously (IV), intramuscularly (IM) or subcutaneously (SC) in 7 sheep. AUC is calculated for each 15 minute time period. Data are presented as mean ± SEM. * indicates $P < 0.05$.

stimulus was unlikely to be a factor because each sheep participated in all 3 studies and were matched against themselves for statistical comparisons between administration routes.

Subcutaneous administration routes are widely considered to provide more variable drug effects than other routes due to their dependence upon the rate of local skin perfusion, which can vary due to changes in temperature or physiological responses such as shock. In the current study, subcutaneous administration of xylazine produced consistent anti-nociceptive effects, although factors such as room temperature and animal handling methods remained constant amongst all sheep. The analgesic profile following subcutaneous injection of xylazine was the flattest of all administration methods, demonstrating slower drug absorption and generally lower end-point current values. The slow onset of xylazine following subcutaneous injection observed in this study was expected, as initial drug distribution is dependent upon the local perfusion of the injection site which is generally lower for this site than injection into the venous circulation or better perfused sites such as the muscle. However, it was expected that due to the lower perfusion of the injection site, subcutaneous administration would produce a considerably longer period of anti-nociception than intramuscular injection. It may be that the dose of xylazine used in this study was not sufficient to provide consistent analgesic effects following subcutaneous injection. The slower drug absorption following subcutaneous injection results in lower systemic drug concentrations, and whilst the drug may still be present systemically and for a long period, the concentrations may not be sufficient to cause useful drug effects. Higher dosing may be required when using subcutaneous injection of xylazine to go above the minimum effective concentration (MEC).

3.4.1 Conclusions

For the treatment of pain from surgical and husbandry procedures the ideal analgesic should provide a relatively rapid onset with a long period of steady state analgesia, the analgesic effect should be predictable, and the administration simple. Intramuscular and subcutaneous injection of xylazine offer very simple methods of administration that provide significant analgesic effects for a longer period than intravenous administration. However, the slow onset and lesser magnitude of analgesic effects, at the dose used in this study, make subcutaneous injection less favourable for the routine treatment of pain.

Intramuscular injection fulfills the greatest number of criteria needed for the routine management of pain in sheep and for studies in this thesis. The time to peak analgesic effects compared favourably with intravenous administration, but with a longer period of significant analgesic effects and less unnecessarily high initial blood concentrations and the consequent risk of sedative effects. The duration of analgesic effect provided by a single intramuscular bolus may not be sufficient for the treatment of all forms of pain and further studies examining alternative preparations and different α_2 agonists may provide dosing strategies that could provide longer term pain control. The use of a bolus intramuscular loading dose followed by continuous infusion of xylazine to provide longer term, steady-state analgesia in sheep is examined in a subsequent chapter.

Chapter 4

The cardiovascular and respiratory effects of low dose intramuscular xylazine in conscious sheep

Data from this chapter has been previously published as : Grant, C. & Upton R.N. (2001) Cardiovascular and haemodynamic effects of intramuscular doses of xylazine in conscious sheep. *Australian Veterinary Journal* **79(1)**, 58 – 60.

4.1 Introduction

As shown in Chapter 3, low dose, intramuscularly administered xylazine demonstrated significant anti-nociceptive effects and appeared a suitable analgesic for the management of pain in sheep. However, in addition to efficacy, an analgesic should be safe; the analgesic should be free from deleterious side effects and should never endanger the well-being of the animal.

Xylazine is widely used by veterinarians as an anaesthetic, analgesic or sedative in many animal species. Dose requirements can vary widely between species, with xylazine being only marginally effective in swine whilst sheep and goats are particularly sensitive to its action (Flecknell, 1987; Maze and Tranquilli, 1991). The cardiovascular effects of xylazine including reductions in heart rate (HR), cardiac output (CO) and transient hypertension followed by hypotension have been consistently described in many different species (Klide et al., 1975; Maze and Tranquilli, 1991; de Segura et al., 1997). However, these side effects have mostly been noted following higher intravenous doses designed to produce profound sedation or anaesthesia, and under such circumstances these effects are usually expected and can be tolerated. When xylazine is administered to provide analgesia in conscious sheep, any undue cardiovascular or respiratory challenge may prove detrimental to the welfare of the animal and have significant consequences for this use of xylazine, particularly 'in the field'.

The purpose of this study was to determine if a low dose intramuscular xylazine regimen designed to provide analgesia for sheep produced detrimental cardiovascular or respiratory changes.

4.2 Methods

4.2.1 Animal preparation

Six sheep were instrumented as described in section 2.3 and were allowed at least 1 week to recover from surgery.

4.2.2 Study design

On the experimental day, the sheep were transported within their metaboilc crates to the experimental room and placed in a sling as described in Section 2.3.4 of the Methods chapter. Sheep were allowed a period of approximately 30 minutes to acclimatise to their surroundings before the commencement of the study.

4.2.2.1 General design

In each study, baseline variables were recorded for 5 minutes and a baseline arterial blood sample taken. Sheep were then administered 2.5 mg of intramuscular xylazine (Troy Laboratories, Smithfield NSW, Australia) in the right hand side rump, prepared in a volume of no less than 5 ml using 0.9% saline. All variables were recorded for a further 60 minutes and blood samples and cardiac output measurements taken as described below.

4.2.2.2 Parameter measurement

For blood pressure measurements, one of the arterial catheters was connected to a transducer (Abbott Ireland Ltd, Slago, Republic of Ireland) and the continuous waveform displayed on a monitor (Hewlett-Packard model 78345A, Boeblingen, Germany). The output from the blood pressure monitor was recorded at 1 Hz using an analog to digital (A-D) data acquisition card (Metrabyte, DAS 16-G2) and a personal computer (Microbits 486 based IBM compatible). Heart rate was determined by counting the number of systolic peaks on the waveform over a 15 second period.

Haemoglobin saturation and carbon dioxide tension were measured in arterial blood using a gas analyser (ABL System 620, Radiometer, Copenhagen, Denmark).

Cardiac output was measured using the thermodilution catheter connected to a cardiac output computer (Model no. 330, Abbott, North Chicago IL, USA). Measurements were made at 5, 10, 15, 30, 45 and 60 minutes post xylazine injection. For each measurement, three separate injections of 10 ml 0.9% saline at 0°C were made into the distal lumen of the thermodilution catheter. The three readings were averaged to give the value for each time point.

4.2.2.3 Blood sampling

Arterial blood samples for the determination of arterial blood gas tensions were taken from the carotid artery catheter at 5, 10, 15, 30, 45 and 60 minutes after xylazine injection. These were collected anaerobically through three-way stopcocks and extension lines connected to the catheter. At the time of sampling, 4 ml of blood was withdrawn into a 5 ml syringe to account for the average catheter-extension line-stop cock dead space plus 1.8 ml. Using a 2 ml syringe containing 25 μl of heparin (1000 IU/ml), a further 0.5 ml of blood was withdrawn. Any air was then expelled from the syringe, the tip sealed, and the syringe was stored at 0°C for up to 30 minutes until gas analysis was performed.

4.3 Data analysis

Blood pressure data acquired from the computerised data acquisition system was stored, along with a corresponding time channel, as tab delimited ASCII data. Python (www.python.org) scripts written by the author were used to extract and average blood pressure values at selected time periods from the raw data files.

4.3.1 Statistical analysis

All values were expressed as the mean and standard error of the mean (SEM). A paired Student's t-test was used to determine the significance of any changes from baseline values, a P value of < 0.05 was regarded as statistically significant.

4.4 Results

The intramuscular injection of 2.5 mg of xylazine did not cause a dramatic change in any of the cardiovascular parameters. Heart rate had a maximal reduction of $8.0 \pm 2.3\%$ at 45 minutes, cardiac output decreased by $13.7 \pm 5.8\%$ at 30 minutes and MAP had a peak reduction of $8.3 \pm 4.9\%$ at 30 minutes (Figure 4.1).

A maximal reduction in PaO_2 (figure 4.2) of $11.7 \pm 3.9\%$ occurred at 30 minutes. This was the only variable measured that achieved statistical difference from baseline. The maximal $PaCO_2$ (Figure 4.2) increase of $6.8 \pm 1.9\%$ also occurred at 30 minutes.

There was no evidence of sedation as evident by an increase in the degree of ptosis, salivation or by a failure to respond to mild auditory or sensory cues during the experimental period.

4.5 Discussion

The administration of an analgesic dose of intramuscular xylazine in sheep failed to produce the substantial haemodynamic or cardiovascular changes often seen in other species (Klide et al., 1975; Haskins et al., 1986; de Segura

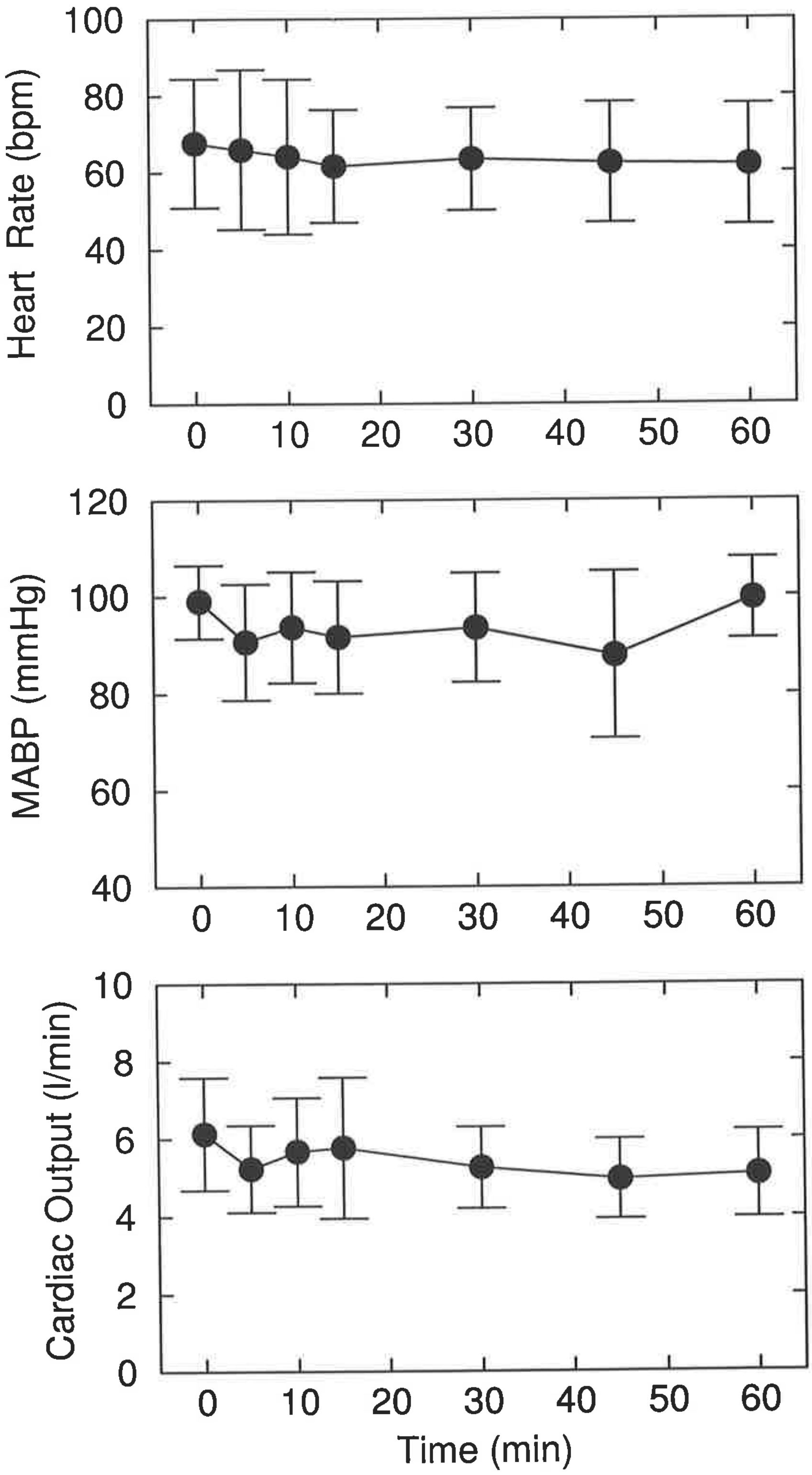


Figure 4.1: Heart rate, mean arterial blood pressure (MABP) and cardiac output following IM administration of 2.5 mg of xylazine. Values are represented as mean and SEM of six sheep.

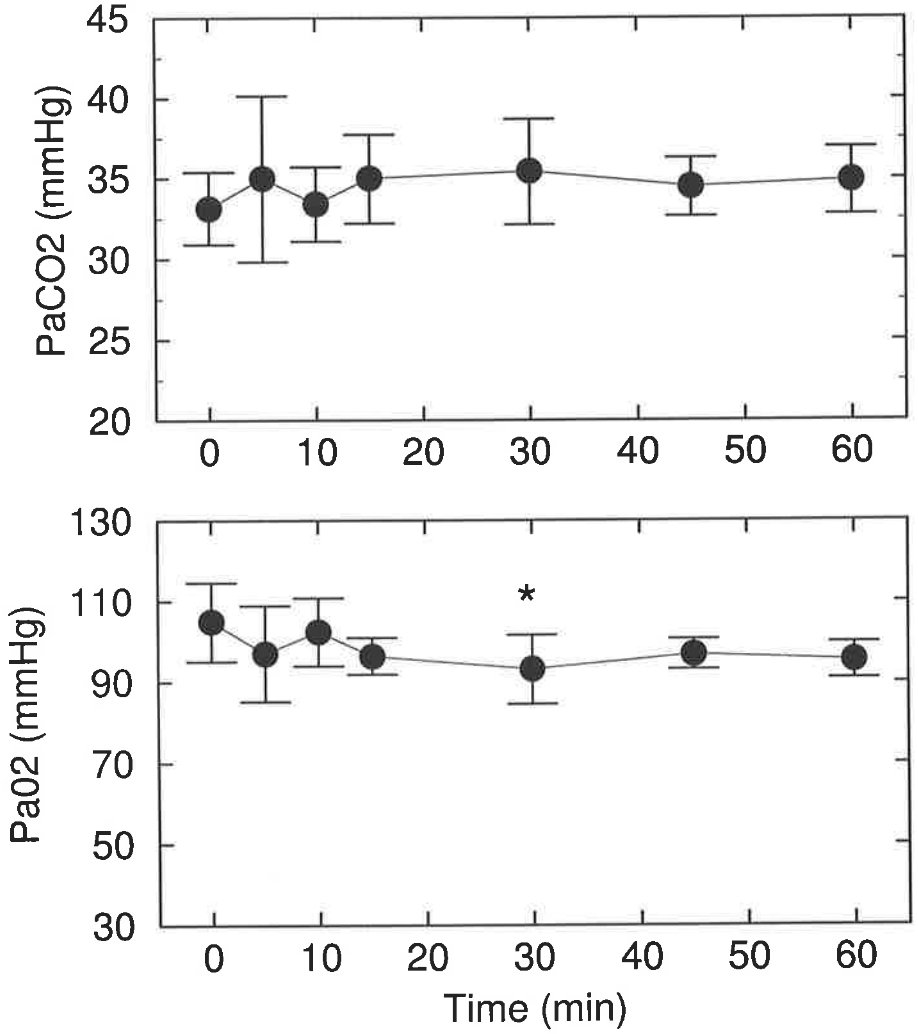


Figure 4.2: Partial pressure of carbon dioxide (PaCO2) and oxygen (paO2) in arterial blood following IM administration of 2.5 mg xylazine. Values are represented as mean and SEM of six sheep.* indicates P <0.05

et al., 1997). The maximal effects appeared to occur by 30 minutes, although this change was only small with arterial oxygen tensions being the only variable to be significantly different from baseline.

Typically, the cardiovascular effects of xylazine administration are described as an initial period of hypertension (5–10 minutes) followed by a longer lasting period of hypotension, a reduction in heart rate due to increased vagal tone and a reduction of cardiac output by one-third to one-half. The reduction in cardiac output is mostly attributed to the reduction in heart rate, the initial hypertension due to xylazine's post-synaptic effects at the adrenergic receptors producing vasoconstriction and smooth muscle contraction (Tranquilli and Maze, 1993). Bradycardyia and arrhythmic effects have also been observed particularly in response to high intravenous doses. These haemodynamic and cardiovascular effects have been observed in horses (Wagner et al., 1991), dogs (Klide et al., 1975; Kyles et al., 1991), cats (Haskins et al., 1975) and pigs (de Segura et al., 1997).

There are a number of factors which may have contributed to the absence of any major cardiovascular changes in response to the administration of intramuscular xylazine in the current study.

The first of these factors is the lower dose used in the present study. Increasing dosage of nearly all anaesthetic/analgesic agents is associated with increasing morbid effects, including changes to the level of sedation, motor function, cardiovascular reflexes and haemodynamic responses. The dosages used in a number of previous studies is in a range commonly used clinically in many species to produce sedation and anaesthesia. However, physiological responses under these circumstances are not necessarily applicable to the lower dosing regimens used in this study and as such some of the cardiovascular drug effects previously reported may be strongly linked to the induction of an anaesthetic state.

The second factor is difference in drug effects and drug sensitivity between species. Ruminants, especially sheep and goats are highly sensitive to the effects of α_2-adrenoceptor agonists requiring a reduction in dose of approximately 20 to 40 times when compared to species such as the cat, dog or horse (Flecknell, 1987). This may be due to variations in the bioavailability of the drug, differing sensitivity at the receptor site or the high degree of inter-species variation in the presence and distribution of α_2-adrenoceptors. Even studies in other species where no sedation or anaesthesia occured, the doses were far higher than the present study. Thus, some of the cardiovascular and haemodynamic responses observed in other studies may not be related directly to the drugs primary analgesic and sedative actions but may be symptoms of higher drug concentrations causing secondary effects to become more evident. For instance, in pigs bradycardia and hypertension followed by hypotension were observed in response to the administration of xylazine, but this was in response to doses far above normal clinical doses (up to 16 mg/kg or 320 times the dose used in this study) with no apparent analgesic effect (de Segura et al., 1997).

While there is a large variation in the analgesic efficacy and dosing requirements of xylazine between some species, the respiratory responses to xylazine administration appear more uniform between the species. In most species, blood gas tensions and pH values remain unchanged, despite reductions in respiratory rate (Haskins et al., 1975; Klide et al., 1975; Wagner et al., 1991). The exception to this pattern of response may be the sheep.

Using the same dosage as this study (50 μg/kg), but administered intravenously as a bolus, Waterman et al. (1987) showed profound decreases in PaO_2 coupled with slight increases in $PaCO_2$ in conscious sheep, effects not generally seen in other species. The presence of circulating α_2 receptors on platelets and subsequent changes to pulmonary blood flow are thought to be responsible for these changes (Eisenach, 1988). Similar but less profound changes were observed in the present study, with rises in arterial $PaCO_2$ and decreases in PaO_2. However, the influence of administration route is important. Intravenous administration of a bolus of xylazine has been shown to cause bradycardia and an immediate increase in systemic arterial pressure followed by a longer lasting reduction in pressure (Bloor and Schmeling, 1993) whereas intramuscularly injected xylazine has a much reduced pressor response due to lower peak blood concentrations and less post synaptic alpha adrenoceptor stimulation (Tranquilli and Maze, 1993). The use of intramuscular administration routes may be a method of achieving acceptable xylazine induced analgesia without producing severe transient hypercarbia or hypoxaemia.

The data from this study verifies the safety of a low dose intramuscular xylazine administration regimen for analgesia in sheep. The slight degree of arterial hypoxaemia observed, whilst statistically significant, was not to a degree that would be a concern in an otherwise healthy animal. However, in a sheep with a severe pre-existing respiratory or cardiovascular disease some degree of caution may need to be exercised. In general, this study supports the use of this dose of xylazine as a safe, effective analgesic for the control of acute pain in the sheep.

Chapter 5

Steady-state analgesia in sheep by continuous xylazine infusion

Data from this chapter has previously been published as : Grant, C., Summersides, G.E. & Kuchel, T.R. (2001) A xylazine infusion regimen to provide analgesia in sheep. *Laboratory Animals* **35**, 277–281.

5.1 Introduction

Providing pain relief for experimental animals is an important issue confronting researchers particularly in light of the growing public awareness and interest in animal welfare practices within the research community.

There are an increasing number of experimental sheep preparations being used by researchers. These preparations offer many benefits, such as, the ability to sample large blood volumes, easy instrumentation of the major organs and blood vessels and a comparable mass to humans. Sheep are also easy to handle and adapt readily to short term indoor housing (Runciman et al., 1984a,b). Unfortunately, methods of providing pain relief for these animals has not kept pace with advances in the technical developments of these preparations. There has been no specific algesimetric testing, or verification in sheep, of protocols designed to provide pain relief following surgical procedures.

The ideal analgesic regimen would involve the use of a readily available agent whose pharmacological properties have been well examined and whose relative cost does not prohibit its use. Its application should be simple and provide effective analgesia with no detrimental side effects, such as sedation or respiratory depression, that could compromise the well-being of the animal.

In many commonly used experimental animals, such as cats and dogs, these criteria are close to being realised and analgesics and associated protocols for use have been extensively validated to provide pain relief following surgical treatment and other painful procedures.

The use of analgesics in sheep however, is a smaller and relatively newer area of study and drugs have often been selected for use based on their effectiveness in other species. Unfortunately inter-species variability in drug effects (Baggot, 1992) means that standard treatments cannot necessarily be assumed to be effective in different species. As discussed in Chapter 1, many commonly used analgesics such as opioids are ineffective in the sheep. From the data in Chapter 3 and Chapter 4, it was clear that intramuscular administration of low dose xylazine significantly increased anti-nociception in sheep without negative side effects such as sedation or adverse cardiovascular changes.

However, Chapter 3 showed this method of bolus xylazine administration only provided a period of significant analgesia for approximately 60 minutes. Repeated bolus dosing at this interval is impractical for anything more than the shortest period of time.

Continuous intravenous infusion of drugs is a method of providing long term steady-state drug concentrations. Unfortunately, the delay required to achieve effective drug concentrations means that initial under-dosing can occur. The use of an initial bolus or loading dose, given prior to commencing the infusion is a simple method of achieving higher drug concentrations more rapidly than by infusion alone (Baggot, 1995).

The aim of this investigation was to determine whether the administration of a set dose of intramuscular xylazine followed by continuous low dose intravenous infusion could provide predictable, effective, steady-state analgesia in sheep. If effective, this protocol could form the basis of a strategy for the longer term management of post-operative pain in sheep.

5.2 Methods

5.2.1 Animal preparation

Six adult male merino sheep between 45 and 60 kg were used. Prior to the study the animals were housed indoors in metabolic crates as described in Section 2.2.1 of the Methods chapter.

Twenty four hours prior to the experiment, a 16G intravascular catheter (Angiocath, Becton-Dickinson Infusion Therapy Systems Inc, Utah USA) was inserted into the right jugular vein under local anaesthesia and kept patent with a heparin lock. On the experimental day the sheep were placed in a sling within their crate which prevented them from lying down but otherwise caused no discomfort. The experimental room was kept quiet and free from distractions during the course of each study and companion sheep were always present to avoid isolation stress in the experimental animal.

5.2.2 Study design

5.2.2.1 Algesimetry

Analgesia was quantified using the general algesimetry techniques described in Section 2.4.1 of the Methods chapter.

5.2.2.2 General design

After a period of at least 5 minutes of stable readings, which was then considered baseline, 5 mg of xylazine (Xylazil-20, Troy laboratories Pty. Ltd., Smithfield NSW, AUS) made up in 2.5 ml of 0.9% saline was injected intramuscularly into the contra-lateral leg to which the algesimetry electrodes were attached. A continuous infusion (Atom Adult/Neonatal syringe pump 1235, Atom Medical Corp., Japan) of xylazine (2 mg/hr) through the jugular catheter was also commenced at this time, and analgesia readings were continued every 60 seconds for the next 90 minutes.

5.2.3 Data analysis

For data reduction, end-point current readings were averaged over 5 minutes to give an average value for each 5 minute period (Figure 5.1, solid circle) all values are represented as mean and standard error of the mean (SEM) (Figure 5.1). Dunnett's test was used to identify which points were significantly different from baseline, a P value of less than 0.05 was considered significant.

To identify the origin and duration of steady-state analgesia a multiple comparison ANOVA was performed using the Newman-Keuls method, assuming a P value of 0.05 as significant (Statistica for Windows 5.1, Statsoft inc, Tulsa, USA). The period when time series data was significantly different from baseline but not significantly different from any of the ensuing points was assumed to be a period of steady-state analgesia.

5.3 Results

The regimen of xylazine bolus followed by continuous infusion caused a significant increase in the threshold current required to produce leg withdrawal. From an average baseline value of 6.17 ± 0.76mA, end-point current gradually increased to a maximum of 17.72 ± 2.53mA or 287% of baseline at 90 minutes (Figure 5.1). All peak current values were significantly higher than baseline from 10 minutes onwards ($P < 0.05$). Multiple comparison ANOVA revealed that there were no statistical differences between any points from 10 minutes onwards, this can be assumed to be a period of steady-state analgesia.

There was a slight increase in the degree of ptosis and salivation in some sheep during the course of these studies, but no signs of heavy sedation were noted. During the periods of highest anti-nociception, no animal failed to respond to mild visual, auditory or other sensory cues. All sheep produced a leg lifting response when gently touched on the foreleg.

One animal was excluded from the data set and the experiment terminated prematurely, due to the animals refusal to elicit a leg lift in response to increases in current.

5.4 Discussion

From the data presented in this study, continuous intravenous infusion of xylazine following an intramuscular loading dose appeared to be an effective and simple method for providing and maintaining analgesia in sheep.

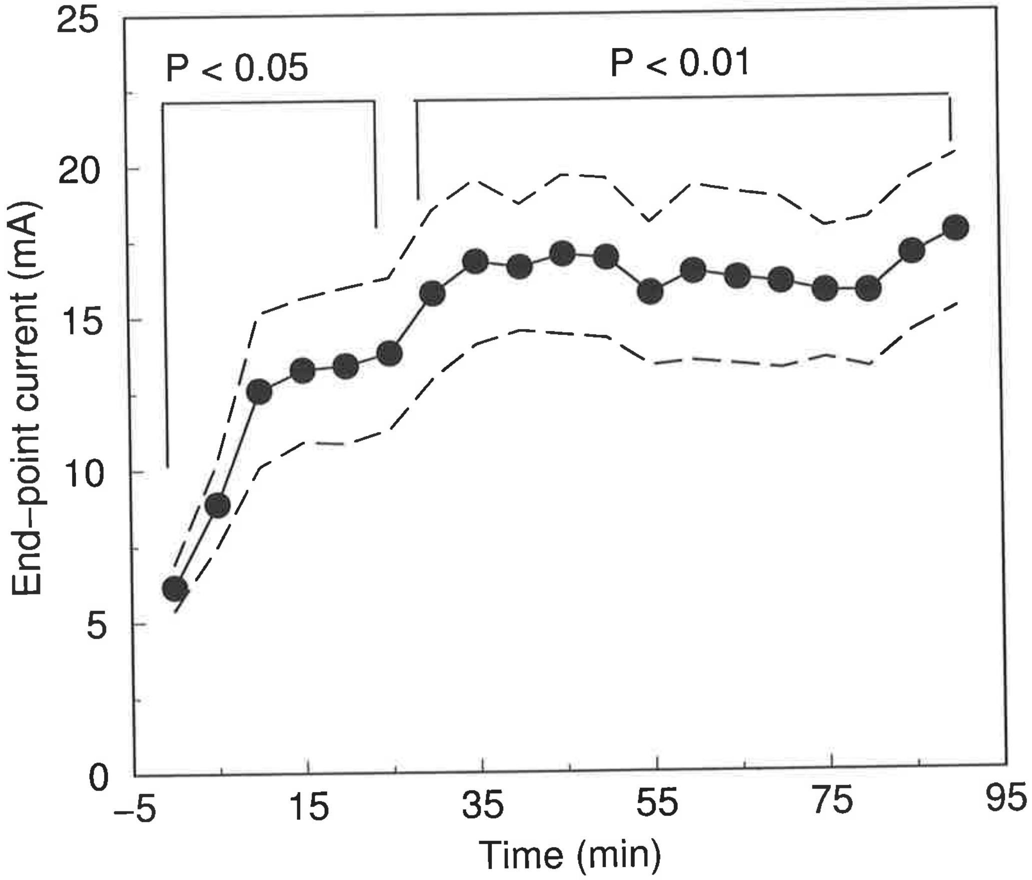


Figure 5.1: Algesimetry changes (mean ± SEM) in response to 5 mg IM xylazine followed by 2 mg/hr continuous IV administration of xylazine. Time 0 represents the time of injection and start of infusion.

The level of analgesia, as indicated by the increase in threshold current rose steadily during the study period until it reached a steady-state at 10 minutes. Although this could be considered an adequate analgesic profile, in some situations an optimal analgesic profile may require steady-state analgesia to be reached sooner than this. One method of achieving faster analgesia would be to increase either the loading dose or the infusion rate. The use of low bolus and infusion doses however reduces the likelihood of the negative side effects associated with increased doses of α_2-adrenoceptor agonists such as sedation, respiratory depression and changes in cardiovascular function (Klide et al., 1975; Waterman et al., 1987; Celly et al., 1997). The bolus was intended to provide steady-state analgesia faster than could be achieved by continuous infusion alone. Increasing the bolus dose, whilst reducing the time to steady-state analgesia, may also leads to an unnecessary overshooting of peak xylazine concentrations and increasing the risk of unwanted side effects. An argument could also be made for administering the loading dose prior to commencement of surgery to obtain any possible benefits associated with pre-emptive analgesia. Opinion is divided on the value of pre-emptive analgesia but there is evidence that the presence of existing chronic pain may reduce the analgesic efficacy of xylazine (Ley et al., 1991).

As discussed in Section 2.4.2 of the Methods chapter, doses were administered based upon the average weight of the Merino sheep used in experimental practice, rather than normalising the dose based upon the mass of the sheep. Using a set dose in this manner provided a simple method of administration and resulted in a consistent analgesic profile amongst the sheep (Figure 5.1). However, for animal groups with a large variation in body weight it may be advisable to adjust doses according to body weight and administer drugs in terms of dose per kg. Expressing the dose regimen of the present study in such terms, and assuming a 50 kg sheep, this equates to a loading dose of 100 μg/kg and an infusion rate of 40 μg/kg/hr.

The point at which the sheep elicit a leg lifting response and terminate the application of current is entirely at the discretion of the sheep. Unlike some algesimetry methods that use a set level of stimulus and escape time latency as a point of reference, the ramped stimulus ensures that the animal dictates it own "comfort zone" and at no point during the study could the sheep be considered to be suffering. This self-determination is exemplified by the refusal of one sheep in the experimental group to lift its leg in response to the painful stimulus. This behaviour is not entirely unexpected as the leg lifting response used in this study is not a reflex but a cognitive voluntary behaviour and as such individual animals occasionally engage in displays of defiance. Testing of the device on the authors showed that the end-point current at which the sheep responded, in the control phase, could be equated to a mildly uncomfortable sensation. The 15 mA threshold reached by all sheep in response to the regimen described was too painful for the unmedicated authors to bear.

The use of bolus doses of xylazine as an effective analgesic agent in sheep has been reported before (Flecknell, 1987) but due to the variability in effect (Ley et al., 1990) and duration of action (approximately 60 minutes) (Nolan et al., 1987a) it has not been considered ideal for long term pain control. The results of this study indicate that the concept of a loading dose of xylazine followed by continuous infusion can provide effective, predictable steady-state analgesia in the sheep. This may provide researchers and animal carers with a simple technique for the control of longer term pain in sheep.

Chapter 6

The anti-nociceptive efficacy of low dose intramuscular xylazine in lambs

Data from this chapter has previously been published as : Grant, C. & Upton, R.N. (2001) The anti-nociceptive efficacy of low dose intramuscular xylazine in lambs. *Research in Veterinary Science* **70,** 47–50.

6.1 Introduction

In Chapter 3 it was shown that a single, intramuscular injection of the α_2-adrenoceptor agonist xylazine produced a significant anti-nociceptive effect in adult sheep. This method was extended to develop xylazine regimens for the control of post-operative pain in adult sheep (Chapter 5). However, the largest welfare issue for sheep is husbandry practices in lambs. Approximately 30 million lambs undergo routine husbandry procedures such as tail docking, castration and mulesing in Australia each year. The administration of low dose intramuscular xylazine may provide a simple method for achieving effective pain control and creating welfare improvements for this large population of animals that remain under-represented in discussions of animal welfare issues.

However, not only can there be a variability in drug effects between species (Baggot, 1992; Riviere et al., 1997) and even breeds (Ley et al., 1990), the pharmacokinetics and action of many drugs often differs between adults and neonates. Drug doses based on body-weight that may be efficacious in adults can display different actions in neonates. This can be due to factors such as the lack of development of the biotransformation pathways associated with drug metabolism and inefficient renal function, which alter the normal half-life of the drug (Baggot, 1995). Factors of scale also need to be considered. Drug dosing requirements correlate not with bodyweight, but with surface area, that varies as the two-thirds power of body weight (Dedrick, 1973). The large variation in bodyweight between adult sheep and lambs may result in the under-dosing of lambs when administered drugs based on a set dose per kg.

The aim of this study was to examine the anti-nociceptive effects of low dose xylazine in lambs with respect to its potential for providing pain relief during painful husbandry procedures, and to also determine if any variation in effect exists between lambs and adults of the same breed but greatly differing age and body weights.

6.2 Methods

6.2.1 Animal preparation and handling

Algesimetry studies in lambs were performed in the sheep handling yards at the Gilles Plains field station of the Institute of Medical and Veterinary

Sciences. Merino lambs (n=7) of 4–6 weeks of age with a weight of 12.8 ± 2.0 kg (mean ± SD) were used.

Ewes and their lambs were held in a pen approximately 6 x 6 m square. To avoid isolation stress or separation anxiety in the lambs, lambs were placed in a sling in a corner of the pen. The sling prevented them from lying down or engaging in locomotory activity but otherwise caused no discomfort.

6.2.2 Algesimetry methods

Nociceptive thresholds were measured using the general algesimetry methods described in Section 2.4.1 of the Methods chapter. The operator however, was situated outside the pen so as not to disturb normal interactions between the lamb, it's mother and other sheep. Readings were taken every 60 seconds, the ramp time for each reading was approximately 5 seconds.

6.2.3 General design

After a period of at least 5 minutes of stable algesimetry readings, that was then considered baseline, an intramuscular injection of 50 μg/kg of xylazine (Xylazil-20, Troy Laboratories, Smithfield NSW, Australia) was administered via the contra-lateral leg. Readings were continued every 60 seconds for the next 60 minutes.

6.2.4 Data handling

The algesimetry data from the lambs was compared to previously published data examining the same dose rate in adult Merino sheep (n=6) of 1.5 to 3 years from the same blood line as the lambs (Grant et al., 1996). These animals had an average weight of 53.4 ± 9.6 kg (mean ± SD) and received the same nociceptive testing and analgesic treatment as the lambs.

6.3 Data analysis

To determine any statistically significant change in the anti-nociceptive response of the lambs, the 95% Confidence Intervals (CI) for the difference between the means of the baseline data and time points following xylazine administration were calculated. If the upper and lower limits of the 95% CI did not include 0 there was assumed to be a significant difference from baseline at that time point. The two groups were assumed to have unequal numbers of data points (Motulsky, 1995).

A two way analysis of variance (ANOVA) with repeated measures was used to determine any difference in anti-nociceptive response between lamb and adult sheep groups at each time point. The mean anti-nociceptive response to the xylazine administration for the entire time period was calculated by averaging all readings post-xylazine administration. The total anti-nociceptive response was determined by measuring the area under the curve between the time of injection and the 60 minute time point using the trapezoidal rule. T-tests were used to assess any difference in mean and total anti-nociceptive response (AUC) between lambs and adult sheep. A P value of less than 0.05 was considered significant.

6.4 Results

All end-point current values were expressed as (mean ± SEM). From an average baseline end- point current of 5.8 ± 0.72 mA the end-point current

	Lambs	Adults	P Value
Total anti-nociceptive response (AUC, mA x minutes)	584.25±51.55	582.33±168.74	> 0.05
Mean anti-nociceptive response (mA)	9.24±1.67	9.47±2.50	> 0.05
Baseline average (mA)	5.85±0.72	4.52±1.47	< 0.05
Weight (kg)	12.80±0 .77	53.42±3.93	< 0.001

Table 6.1: Comparisons of weight and anti-nociceptive response to 50 ug/kg intramuscular xylazine in lambs and adult sheep (mean ± SEM). Mean anti-nociceptive response, is the average level of current required to elicit a leg lifting response for the 60 minute experimental period following xylazine administration.

increased to a peak value of 13.7 ± 1.49 mA at 21 minutes, and remained above baseline (average 10.1 ± 0.95 mA) for the duration of the experimental period (Figure 6.1). All readings 5 minutes post xylazine administration were statistically different from baseline. No visible signs of sedation such as ptosis or reduced alertness were noted. Even at the highest levels of anti-nociception, all lambs responded to novel stimuli such as touch or noise.

On average, the time required for lambs to start producing consistent responses, that could then form the baseline data, was 3 minutes. During the study in adult sheep, the average time to the first baseline reading was 10 minutes (Grant et al., 1996).

Two way ANOVA revealed no differences between adult and lamb groups to this dose of xylazine at any time period. T-tests showed there were also no differences in mean and total anti-nociceptive response between adult sheep and lambs (Table 6.1). The difference in bodyweights between the two groups was highly significant, there was also a significant difference in the baseline end-point current values between adult sheep and lambs (Table 6.1).

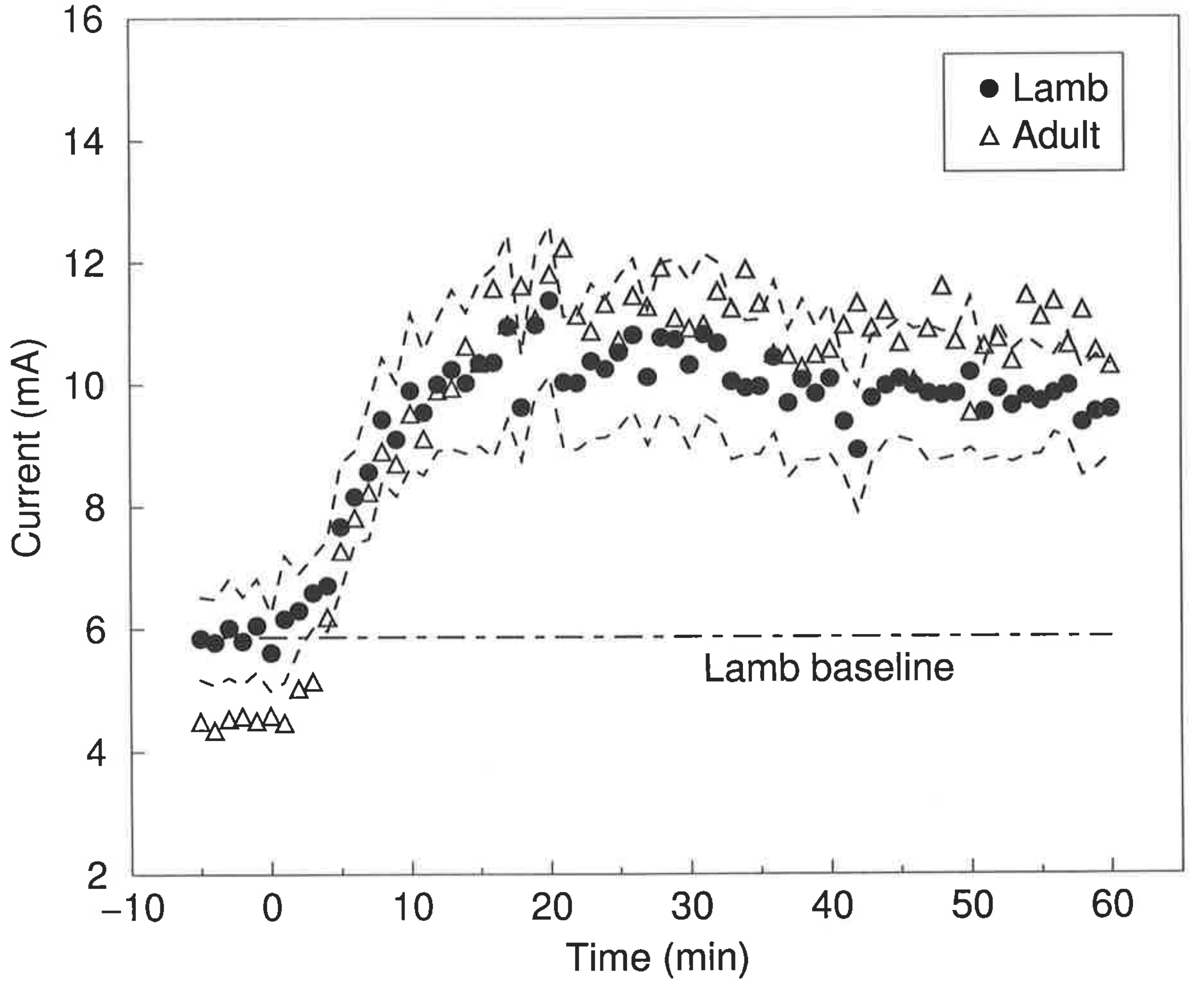


Figure 6.1: The analgesic effect of 50 ug/kg intramuscular xylazine. Mean end-point current values for lambs and adults are shown, the short dashed lines represent SEM for lambs. Average pre-treatment baseline value for lambs is shown as a long dashed line. The vertical axis represents the amount of current required to elicit a leg withdrawal response.

6.5 Discussion

The administration of low dose intramuscular xylazine in lambs caused a significant increase in anti-nociception. From experience and trials on the author, the baseline current values (5.8 ± 0.72 mA) represented a mildly uncomfortable sensation and maximal current values (10.1 ± 0.95 mA) could be considered moderately painful (Ludbrook et al., 1995). This change in response to the electrical stimulus was not induced by inhibited locomotor activity or depression of central reflexes, as there were no signs of sedation.

Despite significant differences in age and bodyweight between the two groups, the anti-nociceptive response of the lambs was similar to that previously reported in adult sheep (Grant et al., 1996). There appeared to be no variation in the anti-nociceptive action of xylazine due to bodyweight related scaling effects. The difference in end-point current values between adults and lambs during the baseline period is due to a single sheep from the adult group. This sheep initiated the leg withdrawal response at a particularly low end-point current (0.6–0.9 mA). If this animal is excluded from the combined data set the adult sheep baseline values are not statistically different from those of the lambs.

In many animals the pharmacokinetics of drugs can vary with age. Neonates may display reduced drug responses due to inadequate development of metabolic pathways and inefficient renal function. This development is rapid in the first 3–4 weeks then slowing up until the tenth week post partum (Baggot, 1995). In the rat there is some evidence of a variation in the anti-nociceptive effect of xylazine due to such factors. Adult rats receiving xylazine exhibit anti-nociceptive effects in response to a formalin induced pain test (Abbott and Bonder, 1997), while infant rats show no evidence of analgesia in response to a similar testing regimen (Yi and Barr, 1996). The lambs used in the present study could be considered to be substantially advanced along the developmental time-line. Lambs were chosen at this age because this is the age at which husbandry procedures are generally performed in Australia. However, in the United Kingdom these procedures are usually performed at a much younger age due to legislative requirements. It is possible that different effects may be observed if a similar study were performed in 1 week old lambs.

Another finding of the current study was the difference in the rate of learning of the algesimetry method between adults and lambs. Because the nociceptive response to the electrical stimulus used in this study is not an involuntary reflex but a learned cognitive behaviour, a period of training can be required until the sheep yield consistent baseline analgesia readings (Ludbrook et al., 1995). In adult sheep this period was variable but on average took 10 minutes, with approximately 1 in 20 sheep failing to ever produce any reliable readings. The stereotypical behaviour of refusing to elicit a leg lift in response to increasing current is an example of a non-adaptive response and is unrelated to the painfulness of the stimuli. This behaviour is common in learned response models of pain measurement that incorporate higher levels of function in the central nervous system regarding pain, rather than a simple reflex response (Lineberry, 1981).

During the present study in lambs, the training period required was dramatically reduced to 3 minutes, with some lambs' second reading forming the first baseline data point. No lambs were excluded on the basis of failure to "train up". This is similar to previous findings, that in contrast to adults, lambs required no training to produce steady baseline threshold values in response to a mechanical nociceptive algesimetry test (Thornton and Waterman-Pearson, 1999). It is evident from these two different algesimetry testing methods that lambs could provide an excellent model for investigations involving nociceptive responses.

From the data presented in this study it is clear that the intramuscular administration of low dose xylazine to lambs produced a significant degree of anti-nociception and that this response was similar in all respects to the effect seen in adult sheep of much greater body weight. The confirmed analgesic efficacy and absence of negative side effects such as sedation suggest this regimen has potential for relieving pain in lambs subject to routine husbandry procedures such as Mulesing, tail docking and castration. This will be examined in detail in a subsequent chapter.

Chapter 7

Behavioural responses of lambs to common painful husbandry procedures.

7.1 Introduction

Algesimetry techniques, as used in Chapters 3 and 6, are useful in describing the anti-nociceptive actions of a drug in a laboratory setting. However, the absolute test of efficacy for any analgesic regimen is its ability to relieve the 'real world' pain for which it was designed. Before an analgesic such as xylazine can be recommended for the reduction of the pain of husbandry procedures in lambs, proof of analgesic efficacy is required.

Unfortunately, obtaining objective measures of pain or pain relief, even from humans, is difficult. Pain is a sensory experience that for a given a set of noxious stimuli can elicit many different descriptions of the pain experience between different human subjects. Pain can also elicit an emotional response that can interact with the sensory experience of pain. This leads to a wide variation in the description of painful experiences from human subjects. In animals, the situation is further complicated as it is not possible to obtain verbal reports of the experience. Whether animals experience pain with the same qualitative and affective responses as humans is unknown.

As discussed in Section 1.2.4.1 of Chapter 1, the recording of active pain behaviours (APBs) and postures in lambs can provide an objective measure of the painfulness of some husbandry procedures. These techniques have been used to investigate the painfulness of castration and tail docking by burdizzo, a device that crushes a section of the tail to prevent blood flow to of the tail, and tight rubber rings (Kent et al., 1995; Molony et al., 1993, 1997). A good index of pain can also serve as an index of analgesia, and these techniques have also examined the influence of local anaesthetic on reducing these painful behaviours following castration and tail docking (Wood et al., 1991; Kent et al., 1998; Graham et al., 1997).

However, the painful treatments examined in these studies mostly generated similar pain types as a result of ischaemia caused by the application of tight rubber rings. Their usefulness in defining the pain response from other husbandry procedures such as Mulesing had not been verified. Furthermore, it is not known if the behavioural and postural responses elicited by different pain types such as the surgical pain from Mulesing and the ischaemic pain caused by rubber rings are directly comparable.

The aim of this chapter was to determine if the existing behavioural and postural techniques of pain measurement in lambs were applicable to these procedures.

This chapter describes:

1. The general methods used to assess pain by means of behavioural and

postural indicators.

2. Computer software developed for the recording and analysis of these behaviours.

3. The use of these indicators in assessing the painfulness of different husbandry procedures.

7.2 Methods

7.2.1 Behavioural indicators of pain

The behavioural indicators of pain used in this study were based on successful criteria used in previous studies of acute pain in lambs following husbandry procedures (Mellor and Murray, 1989b; Molony et al., 1993; Kent et al., 1995; Graham et al., 1997; Kent et al., 1998). The behavioural and postural indices used in this chapter are listed below. The 90 minute experimental period was divided into 1 minute observation periods and the occurence or count of each behaviour or posture was recorded for each 1 minute period.

7.2.1.1 Active pain avoidance behaviours

Active pain avoidance behaviours are behaviours or responses to pain that in many cases appear to have no direct beneficial effects to the lambs, although they can generally be regarded as attempts to escape the painful stimuli. The active pain avoidance behaviours listed below are known to be exhibited by animals in pain but may also be part of the animals normal behaviour pattern. Therefore, some of these 'pain' behaviours may be observed in lambs free from pain. However, following painful husbandry procedures the incidence of these behaviours can increase until all of the lambs time is spent in 'abnormal' active behaviour.

Vocalisation Although a normal behaviour, vocalisation is also a sign of distress in lambs. It is typically displayed when a ewe and her lamb are separated; both animals will bleat until they are brought together again. However, the frequency of vocalisation of the lamb rapidly decreases as the lamb remains in isolation, and adapts to the situation (Hulet et al., 1975). For this reason, whenever possible, lambs were observed in pens with their mothers and were given time to adapt to this environment before observations commenced. The actual score used was a frequency count and represented the number of distinct vocalizations during each 1 minute observation period.

Restlessness Restlessness is a measure of the lambs inability to attain a comfortable posture and records the number of times a lamb stood up and lay down. Each event of getting up and laying down was scored as a single value.

Tail wagging A normal behaviour in lambs, particularly during suckling, but can also be indicative of pain from caudal areas in the lamb. Each distinct tail wagging period, was considered a single value. These events usually lasted less than 10 seconds and were qualified as being separated from each other by at least 3 seconds.

Kicking/Foot Stamping Each individual kick of the rear legs or stamp of the front foot was counted, these events occured in either standing or lying postures.

Rolling The number of events where lambs lying on the ground rolled over from a lateral position to a supine position, or contra-lateral position, were recorded.

Jumping The number of individual jumps or instances of bucking by the lamb were counted for each time period. These were distinguished from kicking events by the lamb having two or more legs off the ground at the one time.

Licking/Biting Licking or biting a wound site can represent attempts by the lamb to alleviate a painful area by sympathetic stimulation of receptors around the wound site.

Hyperventilating Panting or hyperventilating can, but not exclusively, occur as a result of physiological response to pain. The occurence of panting by the lamb was noted for each 1 minute observation period.

Trembling Trembling can be noted in response to pain or as a result of fatigue due to active behaviour exhausting reserves of energy. The occurence of trembling by the lamb was noted for each 1 minute observation period.

Stretching Stretching can be seen as a normal behaviour but can also be seen as an attempt to reduce pain by stimulating non-painful nociceptors. The occurence of stretching by the lamb was noted for each 1 minute observation period.

7.2.1.2 Postural indicators of pain

Postural changes can be voluntary or involuntary. Involuntary postural changes can be initiated by spinal and brainstem reflexes causing hyperflexia of the limbs (Molony et al., 1993). This can be seen as a full extension of the hindlimbs or changes in gait. Voluntary changes in posture can be a reflection of attempts to allieviate pain by reducing stimulation to painful areas, seen as immobility or statue standing. The postures recorded were broadly divided into 2 groups, standing or recumbent, and within in each of those groups were classifications ranging from normal postures often seen in lambs, to grossly abnormal postures, rarely seen in normal lamb behaviour. The posture adopted by the lamb for the majority of the 1 minute observation period was therfore recorded as one of the following.

Standing postures

Normal standing/walking (S1) Lamb is upright and standing or moving freely with normal levels of alertness.

Slightly abnormal standing/walking (S2) Abnormal walking or standing with barely detectable, swaying, ataxia or abnormal stance.

Extremely abnormal standing/walking (S3) Grossly abnormal standing or walking. Walking on knees, stilted gait etc.

Statue standing (SS) Immobile standing with an obvious loss of interaction with other pen members and outside stimuli. As a way of minimizing stimulation to painful areas lambs can enter a state of reduced alertness and locomotion. In previous studies Statue Standing was classified into two separate groups, SS1, immobile standing for >10s and SS2 immobile standing for > 20 seconds. Because periods of immobility were able to be calculated from travel data supplied by the pen map (Section 7.2.1.3), the criteria for SS was not only the immobility of the lamb but also an obvious withdrawal from interaction with other pen members and outside stimuli.

Easing quarters Hind limbs are bent to minimize stimulation in rear quarters, in recumbent position rear is held off ground.

Recumbent postures

Normal lying - head up (V1) Ventral lying with front and rear legs tucked underneath. Lamb awake, head up and alert.

Normal lying - head down (V2) Posture as for V1 but head down, lamb may be asleep.

Abnormal lying - partial leg extension (V3) One or both hindlimbs partially extended from normal ventral position.

Abnormal lying - full leg extension (V4) One or both hindlimbs fully extended. Front legs in normal ventral position.

Lateral lying - head up (L1) Lamb lying on side with shoulder on ground and head up.

Lateral lying - head down (L2) As for L1 but head is down.

7.2.1.3 Locomotion

An inhibition of locomotion may be seen as attempts by the lamb to minimize the stimulation of painful areas, whilst increases can reflect a higher degree of agitation.

Travel For each 1 minute recording period the position of the lamb in the pen was recorded. From this the number of movements and the total and cumulative travel distance for the experimental period could also be calculated.

7.2.2 Behavioural recording software

The behavioural and postural indicators of pain described in Section 7.2.1 and 7.2.1.2 were based upon indices used by other researchers. The data gathering techniques previously used by other researchers were paper based and relied on observers recording lamb behaviours and postures from outside the pen as they occured. The effort involved in then transcribing this data to a form where the data analysis can easily be performed by computer would be considerable, particularly for large numbers of studies.

In this thesis, a computerised method of data collection was developed which made it possible to acquire more indices in a single experiment than in previous studies. This gave greater scope for determining which indices or combination of indices were important for the clinical assessment of pain.

The development of a software solution for recording this data directly to computer for ease of data handling and manipulation was important to the success of this thesis. The software was developed using the Python (http://www.python.org) programming language. Python is an interpreted, interactive, object-oriented programming language with a clear syntax and support for extensions such as graphical toolkits and databases. It runs on various UNIX platforms, Windows, DOS, OS/2, Mac and Amiga. A full printout of the code is give in the Appendix Section 10.1.

7.2.2.1 Software general design

The criteria for the software package were:

1. An easy to use graphical interface for recording the behavioural and postural indices, specific comments and animal locations for each time period.

2. The data output from the program should be stored in a simple ASCII character format for ease of import into secondary analysis programs such as spreadsheets and relational databases.

To best describe the software use and features, the general procedure for recording behavioural observations using the software is given below.

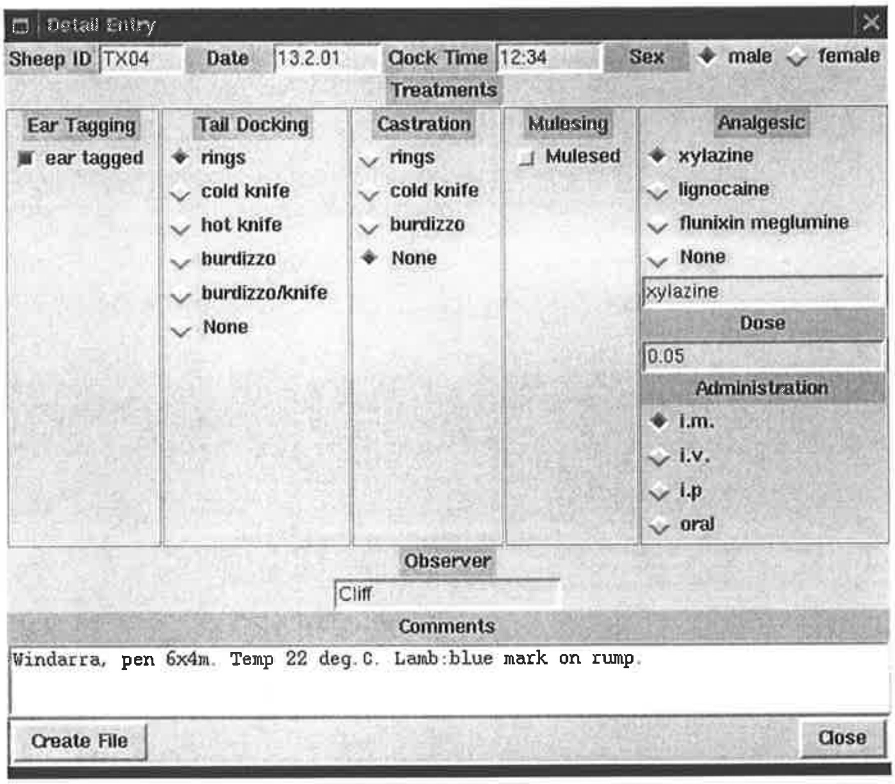
Create New Experiment

To begin recording observations ’New Experiment’ from the File menu in the main window is selected. This opens a Detail Entry window (Figure 7.1) and sheep and experimental details are entered. The ’Create File’ button selects the directory which the data is saved to, the data is saved to a file with a unique filename which is based upon the sheep ID, date and experimental procedure.

Setup of Display

Under the ’Options’ menu in the main window ’Display Setup’ is selected. The Setup window (Figure 7.2) contains various entries that affect the behaviour of controls within the main window. The length of time displayed on the time slider can be altered to reflect the experiment duration. The x and y axes of the travel map are set to the dimensions of the study pen. The ’real world’ clock time can be set to display actual clock time during the experimental period to aid synchronization of observation time periods. The length of each observation period can be set so the time automatically increments by that given value (1 minute for these studies), the alarm can be set to sound when this time period has elapsed since the last entry.

In the main window right clicking within the travel map, located in the lower left hand corner (Figure 7.3), selects if the lambs’ mother was present during



Sheep ID: TX04
Date: 13.2.01
Clock Time: 12:34
Sex: ● male ○ female

Ear Tagging: ☒ ear tagged

Tail Docking: ● rings ○ cold knife ○ hot knife ○ burdizzo ○ burdizzo/knife ○ None

Castration: ○ rings ○ cold knife ○ burdizzo ● None

Mulesing: ☐ Mulesed

Analgesic: ● xylazine ○ lignocaine ○ flunixin meglumine ○ None

xylazine

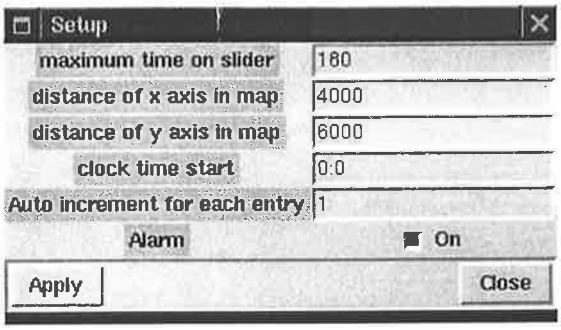
Dose: 0.05

Administration: ● i.m. ○ i.v. ○ i.p ○ oral

Observer: Cliff

Comments: Windarra, pen 6x4m. Temp 22 deg.C. Lamb:blue mark on rump.

Figure 7.1: Screenshot showing New Experiment window. Experimental details are entered and stored at the head of data file.



maximum time on slider 180

distance of x axis in map 4000

distance of y axis in map 6000

clock time start 0:0

Auto increment for each entry 1

Alarm ■ On

Figure 7.2: Screenshot showing the Setup window. Values for travel map size and the behaviour of various controls in the main window can set here.

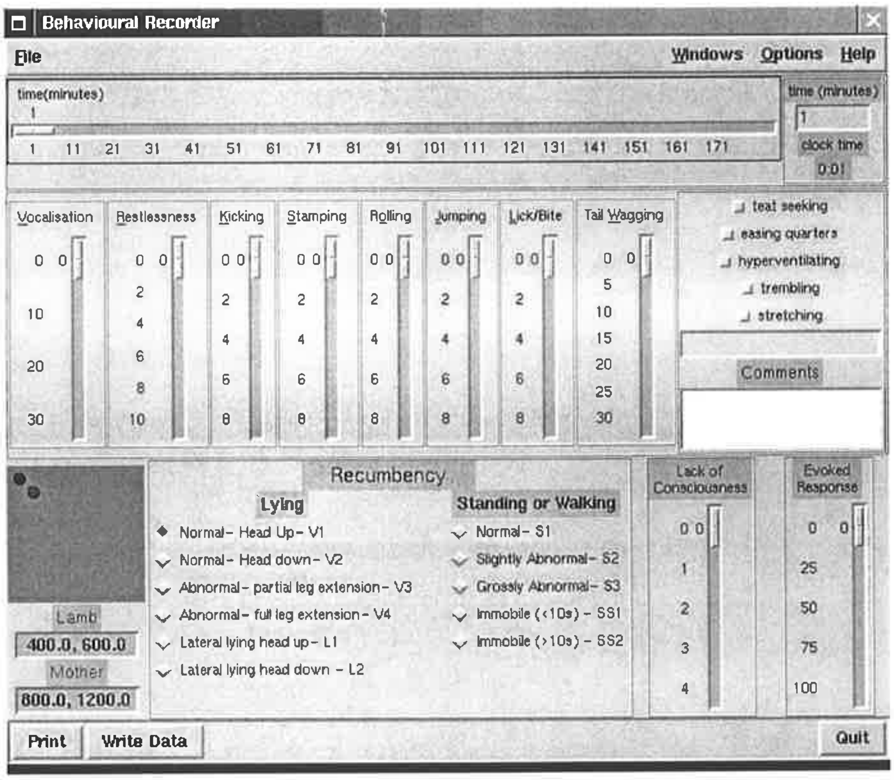
the study.

Recording of behaviours

The main window where all behaviours and postures are recorded is shown in Figure 7.3. In the main window each of the APB's that occur as frequency counts are represented by a slider entry. These can be incremented as they occur by use of the mouse pointer on the slider or incremented by a single value for each press of a hot key. The hot keys for each behaviour are indicated by the underlined letter in the label, eg. vocalisation is 'V', a tail wag 'W'. APBs that are noted just once per time period are recorded via checkbox entries, unlisted APBs may be typed into the entry box below the list. Comments particular to the given observation time period can be also entered.

The location of the lamb and its mother (if applicable) within the study pen are recorded by moving the symbols representative of each to their locations on the travel map, located in the lower left hand corner. The lamb and it's mother are represented by a blue and red dot respectively. Co-ordinates based upon the pen dimension supplied in the Display Setup window are shown below the map.

The posture adopted by the lamb for the majority of the 1 minute



time (minutes): 1

clock time: 0:01

Lying:
- ◆ Normal- Head Up- V1
- ○ Normal- Head down- V2
- ○ Abnormal- partial leg extension- V3
- ○ Abnormal- full leg extension- V4
- ○ Lateral lying head up- L1
- ○ Lateral lying head down - L2

Standing or Walking:
- ○ Normal- S1
- ○ Slightly Abnormal- S2
- ○ Grossly Abnormal- S3
- ○ Immobile (<10s) - SS1
- ○ Immobile (>10s) - SS2

Lamb: 400.0, 600.0

Mother: 800.0, 1200.0

Figure 7.3: Screenshot of the main application window.

observation period is recorded by selecting the appropriate radio button under the Recumbency title.

The 'lack of consciousness' and 'evoked response' measurements were not recorded in this thesis.

The 'Write Data' button at the bottom of the window is used to commit all the variables for each 1 minute time period to the data file.

7.2.3 Animal preparation

7.2.3.1 Animal handling

Behavioural studies were performed at the Institute of Medical and Veterinary Sciences, Windarra Farm, Farrell Flat, South Australia. On study days a flock of ewes with lambs 'at foot', of 4–6 weeks of age and a mean weight of 11.8

± 0.5 kg, were brought into the stock yards. Mother and lamb pairs were identified and marked with scourable stock spray marker. These pairs were then placed in pens of approximately 6 x 4 m, separate from, but alongside the larger flock.

7.2.3.2 Husbandry procedures

The husbandry procedures examined (listed below) were all normal sheep husbandry procedures in Australia and were performed as part of the standard lamb marking procedures of the farm.

Tail docking by rubber rings (RRT) A small latex rubber ring with an inside diameter of approximately 5 mm was placed below the third palpable joint of the tail by means of an elastrator device. It was ensured that the remaining tail length was sufficient to cover the anus.

Tail docking by hot knife (HK) Involved removal of the tail below the third palpable joint by the use of a docking iron or 'hot knife'. Propane gas was used to heat the opposable jaws of the device that were placed either side of the tail. Sufficient pressure was applied to the handles to ensure clean cutting and cauterising of the tail.

Castration by rubber rings (RRC) Latex rubber rings were applied with an Elastrator to the neck of the scrotum. Both testes were trapped within the scrotum distal to the ring.

Mulesing (M) Mulesing is a technique developed by Mr J. H. W. Mules of South Australia for surgically de-wrinkling the fly-strike susceptible breech area of the sheep. It is widely employed in many Australian sheep farms, particularly in areas where the blowfly *Lucilla cuprina* is prevalent. Hand shears were used to strip the skin from the base of the tail to a point on both sides between the anus and the hock, an area of approximately $80cm^2$. As scar tissue from the mulesing procedure heals and shrinks, the bare area around the vulva and anus is enlarged and stretched creating an area devoid of wool.

Ear tagging (ET) Plastic tags indicating the owner and colour coded for year of birth were inserted into the left ear of male lambs and the right ear of female lambs using special pliers, that create a hole in the ear and insert the tag in a single action.

Control Handling (H) Lambs were removed from their mother, weighed, and placed in the lamb marking cradle but received no other treatment.

7.2.4 Study design

7.2.4.1 General design

Lambs were taken from the pen with their mothers, weighed, and placed in a supine position in a 'marking' cradle and restrained by locking their hocks into stirrups on the side of the cradle. From this position husbandry procedures were performed. All husbandry procedures were peformed by experienced and skilled operators. Groups of six lambs received one of the following treaments:

1. control handling only (H)
2. ear tagging only (ET)
3. tail docking by hot knife (HK)
4. tail docking by rubber ring (RRT)

5. castration by rubber ring (RRC)
6. tail docking by rubber ring + castration by rubber ring (RRTRRC)
7. tail docking by hot knife + Mulesing + ear tagging (HKMET)
8. tail docking by hot knife + Mulesing + castration by rubber rings + ear tagging (HKRRCMET)

Based upon data from previous studies, the amount of tissue involved and observations prior to the study, treatments 1 to 6 in the above list represent the treatments in increasing order of severity. It was considered that treatments H and ET were most likely to be similar. The ranking of HKMET and HKRRCMET was uncertain due to a lack of previous experimental data.

Following treatment, lambs were returned to the pen with their mothers, and activities within the pen were recorded via video for 90 minutes. A video camera situated at a slightly elevated angle to include the entire pen within its field of view was used to record all activities within the pen. The video recordings enabled all behavioural observations to be made at a later date and allowed a more thorough scrutinisation of activities than on-site, one-off, real time logging would permit. It also meant many more studies could be completed in a single day resulting in less interruption to normal farm activities. Most farms have only a single annual lambing period so unless lambs are purpose breed for studies, the time window for completing studies of this nature is quite narrow. Video taping also enabled the human presence and activity levels within the experimental area to be reduced thus lessening the risk of altering sheep behaviour.

7.2.4.2 Behaviour measurement

The video tapes were reviewed at a later date using the behavioural recording software described in Section 7.2.2. The software was run on an IBM compatible personal computer running the Linux operating system.

7.2.5 Data analysis

7.2.5.1 Data handling

Data generated by the behavioural recording software for each study was imported into a relational data base (PostgreSQL, http://www.PostgreSQL.org) to facillitate the handling of the large number of variables. A structured query language (SQL) was used to issue commands to the database to extract data that matched specific criteria. The PostgreSQL database was chosen because it is fully SQL compliant and a Python module is available creating an easy interface between the behavioural recording software and database.

7.2.5.2 Statistical analysis

Discriminant analysis was used to determine the most accurate and economical combination of behavioural and postural indices that would best describe the pain response. This gave an indication of the proportion of lambs that could be assigned to their correct treatment group based upon the values of the indices. Indices that effectively describe the painfulness of husbandry treatments involving the use of rubber rings have been previously determined (Molony and Kent, 1997). The accuracy of these indices in describing the painfulness of the husbandry treatments used in the current study was compared with a range of alternative indices.

To determine differences between treatment groups based upon the behavioural or postural values, a Kruskal-Wallis analysis of variance (KW-ANOVA) was performed, alpha was set at 0.05. If KW-ANOVA showed significant differences between the groups post-hoc Mann-Whitney U tests were performed to compare between each of the treatment groups. A P value of less than 0.05 was assumed to be significant. Statistical analysis was performed using the data analysis language 'R' (Ihaka and Gentleman, 1996).

7.3 Results

7.3.1 Validation of indices

Discriminant analysis using a combination of V4 and REQ (the sum of scores over the 90 minute period for reslessness, rolling, kicking/foot stamping and easing quarters) has previously been succesful in assigning 63% of lambs to their appropriate treatment groups (Molony and Kent, 1997). In the current study discriminant analysis using such indices placed 42% of the lambs in their appropriate treatment groups (Table 7.1).s

A better result was achieved using the combination of abormal postures (AbPos, the sum of time spent in S2, S3, SS, V3, V4, L1, L2 postures), travel, immobile periods and RKROLL (the sum of scores over the 90 minute period for restlessness, kicking/foot stamping and rolling) that placed 65% of the lambs in their appropriate treatment groups (Table 7.2). The addition of other indices slightly improved the scoring in the HKRRCMET group but reduced the

	Actual Group							
Assigned Group	H	ET	HK	RRT	HKMET	RRC	HKRRCMET	RRTRRC
H	**6**	6	6	2	6	1	2	-
ET	-	**0**	-	-	-	-	-	-
HK	-	-	**0**	-	-	-	-	-
RRT	-	-	-	**3**	-	-	-	-
HKMET	-	-	-	-	**0**	-	-	-
RRC	-	-	-	1	-	**3**	1	1
HKMRRCET	-	-	-	-	-	1	**2**	-
RRTRRC	-	-	-	-	-	1	1	**5**
No. correct (20)	6	0	0	3	0	3	3	5
Proportion (0.42)	1	0	0	0.50	0	0.50	0.50	0.83

Table 7.1: Discriminant analysis using V4 and REQ. Groups were control handling (H); ear tagging (ET); tail docking by hot knife (HK); tail docking by rubber rings (RRT); tail docking by hot knife plus Mulesing plus ear tagging (HKMET); castration by rubber rings (RRC); tail docking by hot knife plus castration by rubber rings plus Mulesing plus ear tagging (HKRRCMET); tail docking and castration by rubber rings (RRTRRC).

	Actual group							
Assigned group	H	ET	HK	RRT	HKMET	RRC	HKRRCMET	RRTRRC
H	**5**	-	2	1	-	-	-	-
ET	-	**5**	-	1	-	-	2	-
HK	1	1	**4**		3	-	-	-
RRT	-	-	-	**3**	-	1	-	-
HKMET	-	-	-	-	**3**	-	1	-
RRC	-	-	-	1	-	**5**	1	1
HKMRRCET	-	-	-	-	-	-	**1**	-
RRTRRC	-	-	-	-	-	-	1	**5**
No. correct (31)	5	5	4	3	3	5	1	5
Proportion (0.65)	0.83	0.83	0.67	0.50	0.67	0.83	0.17	0.83

Table 7.2: Discriminant analysis using AbPos RKROLL, travel and immobile. Groups were control handling (H); ear tagging (ET); tail docking by hot knife (HK); tail docking by rubber rings (RRT); tail docking by hot knife plus Mulesing plus ear tagging (HKMET); castration by rubber rings (RRC);tail docking by hot knife plus castration by rubber rings plus Mulesing plus ear tagging (HKRRCMET); tail docking and castration by rubber rings (RRTRRC).

overall accuracy.

7.3.2 Active pain behaviours

The occurence of individual APBs following each treatment are shown in Table 7.3. Not shown are hyperventilation or panting which was recorded in only a single lamb and trembling that was not observed during these studies. The summation of scores over the 90 minute study period for restlessness, foot stamping/kicking and rolling, (RKROLL) is shown in Figure 7.4. Treatments

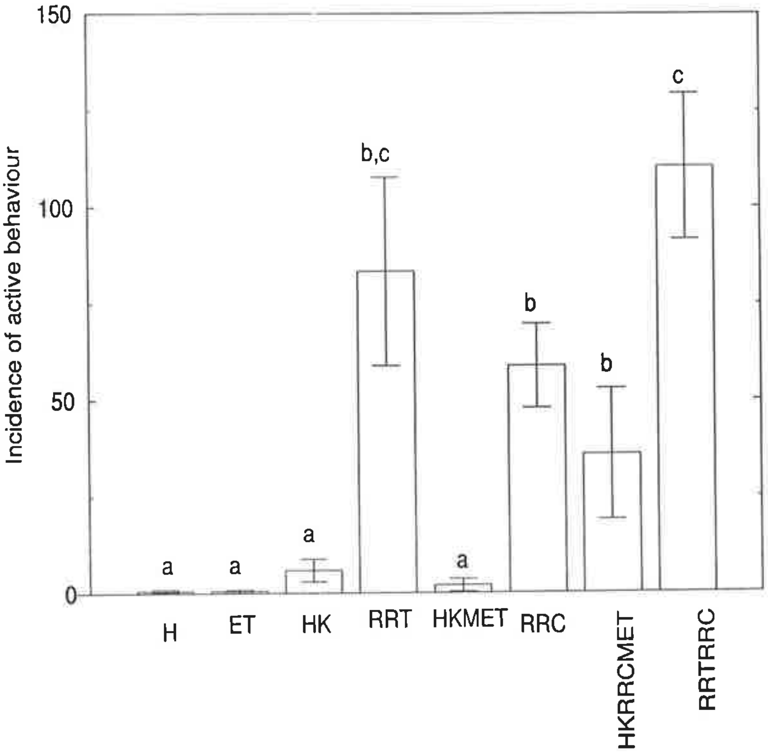


Figure 7.4: The incidence of active pain behaviour (RKROLL) following, control handling (H); ear tagging (ET); tail docking by hot knife (HK); tail docking by rubber rings (RRT); tail docking by hot knife plus Mulesing plus ear tagging (HKMET); castration by rubber rings (RRC); tail docking by hot knife plus castration by rubber rings plus Mulesing plus ear tagging (HKRRCMET); tail docking and castration by rubber rings (RRTRRC) in 6 lambs. (Mean ± SEM). Superscripts indicate treatment groups with a statistically equivalent incidence of active pain behaviour values.

that included the use of rubber ring showed the greatest active pain behaviour responses.

7.3.3 Postures

Time spent in various postures following each of the treatments is shown in Table 7.4. The time spent in abnormal postures showed good correlation with the presumed severity of the treatment (Figure 7.5). Statue Standing was highly indicative of Mulesing treatments, being recorded only in those groups. Ventral lying (V4 and V3) was strongly associated with treatments involving the

APB	H	ET	HK	RRT	RRC	RRTRRC	HKMET	HKRRCMET
kickstamp	0.29±0.29	0	0	10.67± 6.69	14.5± 4.19	30.17±10.23	1.67±1.67	6.67±3.67
roll	0	0	0	1.33±0.89	0	0.5± 0.22	0	0
tailwag	9.14±3.21	4.5± 1.34	0	20.17±13.45	7 3±0.97	24.17±9.31	0	2±1.8
lickbite	0.28±0.18	0.17±0.17	0	5±3.03	0.33±0.21	2.5±2.3	0	0
easquart	0	0	0	0.67±0.49	0.5±0.29	0.33±021	2.5±1.52	7± 3.91
jump	0.43±0.43	0	0.17±0.17	1.83±1.64	0.33±0.33	8.5±5.4	0	0.17±0.17
restless	0.29±0.29	0.5±0.34	5.84±2.98	71.33±24.18	44.33±8.4	79.83±11.52	0.33±0.33	29.33±16.85
vocalise	4.0±3.51	0.17±0.17	1.67±1.67	0.83±0.65	0.33±0.21	23.17±16.9	4.83±2.33	18.33±17.14
travel	55.55±4.75	100.3±19.89	46.2±8.86	39.5±6.22	62.55±12.75	75.5±12.71	39.0±6.2	63.0±14.71
immobile	19.5±3.98	18.17±3.11	25.33±4.13	48.67±5.87	14.33±5.33	9.83±2.06	17.33±9.83	1.33±0.61

Table 7.3: Individual active pain behaviour values following, control handling (H); ear tagging (ET); tail docking by hot knife (HK); tail docking by rubber rings (RRT); tail docking by hot knife plus Mulesing plus ear tagging (HKMET); castration by rubber rings (RRC); tail docking by hot knife plus castration by rubber rings plus Mulesing plus ear tagging (HKRRCMET); tail docking and castration by rubber rings (RRTRRC) in 6 lambs. Values are shown as (Mean ± SEM). See Section 7.2.1.1 for description of each APB.

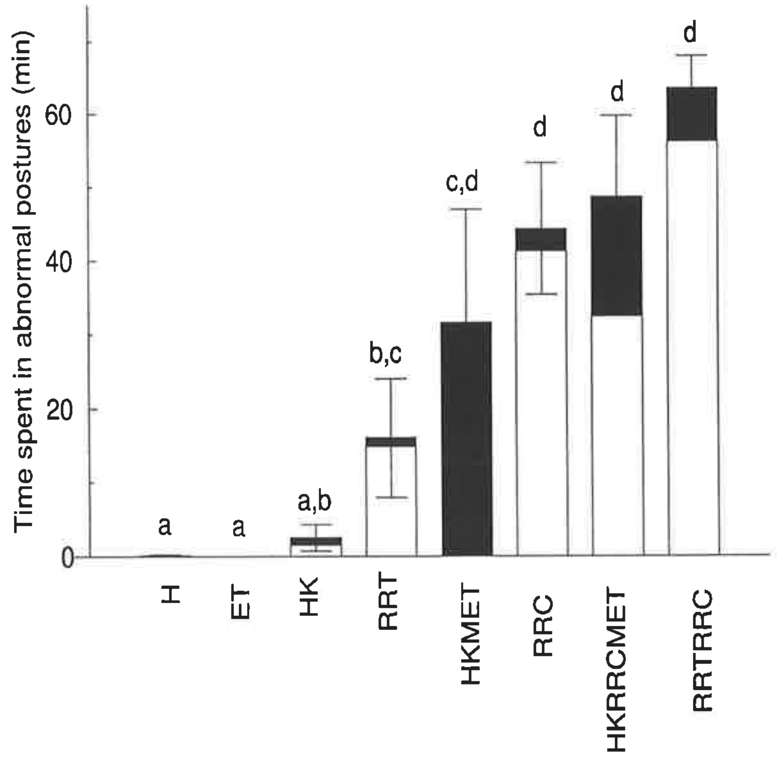


Figure 7.5: Time spent in abnormal postures following control handling (H); ear tagging (ET); tail docking by hot knife (HK); tail docking by rubber rings (RRT); tail docking by hot knife plus Mulesing plus ear tagging (HKMET); castration by rubber rings (RRC); tail docking by hot knife plus castration by rubber rings plus Mulesing plus ear tagging (HKRRCMET); tail docking and castration by rubber rings (RRTRRC) in 6 lambs. (Mean ± SEM). Abnormal standing/walking (■), abnormal lying (□). Superscripts indicate treatment groups with a statistically equivalent time spent in abnormal postures.

use of rubber ring, as was lateral lying (L1 and L2), however the total incidence of lateral lying was low.

7.3.4 Travel

Travel distance for each of the treatments are given in Table 7.3. The number of immobile periods was calculated from the co-ordinates produced by the map entry shown in Figure 7.3. If the distance travelled by the lamb between the 1 minute observations was less than 10 cm, this period was then classified as

Posture	H	ET	HK	RRT	RRC	RRTRRC	HKMET	HKRRCMET
V1	19.14±4.52	1.83±1.83	23.17±8.84	25.0± 4.12	13.33±4.12	9±4.61	2.17±1.38	2.33±1.30
V2	4.57±2.37	0.33±0.33	1.17±1.17	10.33±2.24	0	3±2.24	0	0
V3	0	0	1.5±1.5	9.5±5.01	32.33±7.2	17.33±6.33	0	14.17±8.49
V4	0	0	0	3.33±2.95	8.5±2.95	36.33±6.63	0	18.17±7.94
L1	0	0	0	1±0.52	0.17±0.17	2±1.29	0	0.17±0.17
L2	0	0	0	1±0.82	0.33±0.21	0.5±0.22	0	0
S1	49.14±7.28	78.17±5.68	63.17±9.68	38.67±6.26	27.83±6.29	14.5±5.09	55.67±14.41	37.17±11.6
S2	0.14±0.14	0	1±0.52	1.17±0.98	3.0±1.34	6.67±3.47	4.83±4.06	5.5±3.02
S3	0	0	0	0	0	0.67±0.67	0	0.17±0.17
SS	0	0	0	0	0	0	26.83±16.35	10.5±9.72
Total lying	23.71±4.54	2.17±1.79	25.83±9.86	50.17±6.45	54.67±5.95	68.17±7.7	2.17±9.86	34.83±13.76
Abnormal lying	0	0	1.5±1.5	14.83±8.3	41.33±9.68	56.17±5.24	0	32.5±13.5
Abnormal standing	0.14±0.14	0	1±0.52	1.17±0.98	3±1.34	7.33±3.43	31.67±15.3	16.17±9.19

Table 7.4: Time spent in various postures following, control handling (H); ear tagging (ET); tail docking by hot knife (HK); tail docking by rubber rings (RRT); tail docking by hot knife plus Mulesing plus ear tagging (HKMET); castration by rubber rings (RRC); tail docking by hot knife plus castration by rubber rings plus Mulesing plus ear tagging (HKRRCMET); tail docking and castration by rubber rings (RRTRRC) in 6 lambs. (Mean ± SEM). See Section 7.2.1.2 for a description of each posture classification.

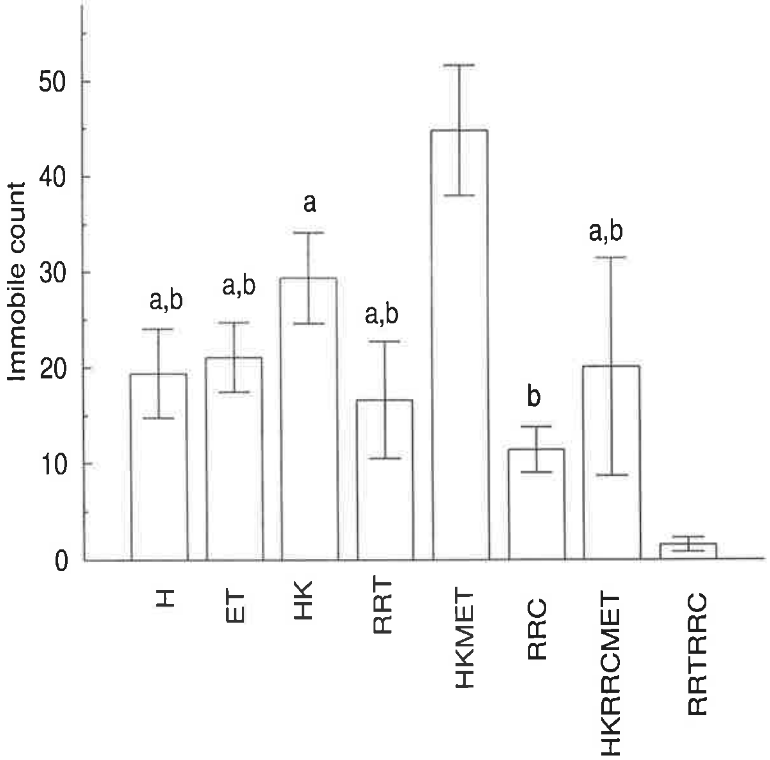


Figure 7.6: Number of periods where lamb was immobile for greater than 1 minute following control handling (H); ear tagging (ET); tail docking by hot knife (HK); tail docking by rubber rings (RRT); tail docking by hot knife plus Mulesing plus ear tagging (HKMET); castration by rubber rings (RRC); tail docking by hot knife plus castration by rubber rings plus Mulesing plus ear tagging (HKRRCMET); tail docking and castration by rubber rings (RRTRRC) in 6 lambs. (Mean ± SEM). Superscripts indicate treatment groups with a statistically equivalent incidence of active pain behaviour values.

an immobile period. The HKMET treatment group showed an increase in the number of immobile periods whilst the rubber ring only treatments (RRT, RRC, RRTRRC) showed reductions in the time spent immobile which was correlated with increasing severity of treatment (Figure 7.6).

7.3.5 Comparison of husbandry procedures

7.3.5.1 Handling, ear tagging and tail docking by hot knife

For the 6 lambs that received handling only (H) there was only one recording of an abnormal posture for the entire study period; the incidence of APB was also low with only 4 events recorded (Figure 7.7).

The ear tag only group (ET) showed no postural or APB changes following treatment when compared with the handling only group (Figure 7.7).

The 6 lambs tail docked by hot knife (HK) showed an increase in the time spent in abnormal postures and an increase in the number of RKROLL behavioural events for the 90 minute study period (Figure 7.7). However, this change was not statistically significantly different from the lambs in the control group (Figures 7.5 and 7.4). Tail docking by hot iron was the only procedure that consistently produced vocalisation in lambs during it's application.

7.3.5.2 Rubber ring treatments

Husbandry methods involving the application of tight rubber rings produced large changes in both behavioural displays of pain and an increase in the amount of time spent in abnormal postures (Figures 7.5 and 7.4). The magnitude of the response correlated with the presumed severity of the treatment giving a ranking where RRT < RRC < RRTRRC .

Tail docking by rubber rings (RRT) produced significant increases in abnormal posture displays and active pain behaviours for 40 - 60 minutes following ring application (Figure 7.8).

Castration by rubber ring also produced large increases in active behaviours and significantly more abnormal postures (Figure 7.5) that took longer than than the RRT group to return to normal values (Figure 7.8).

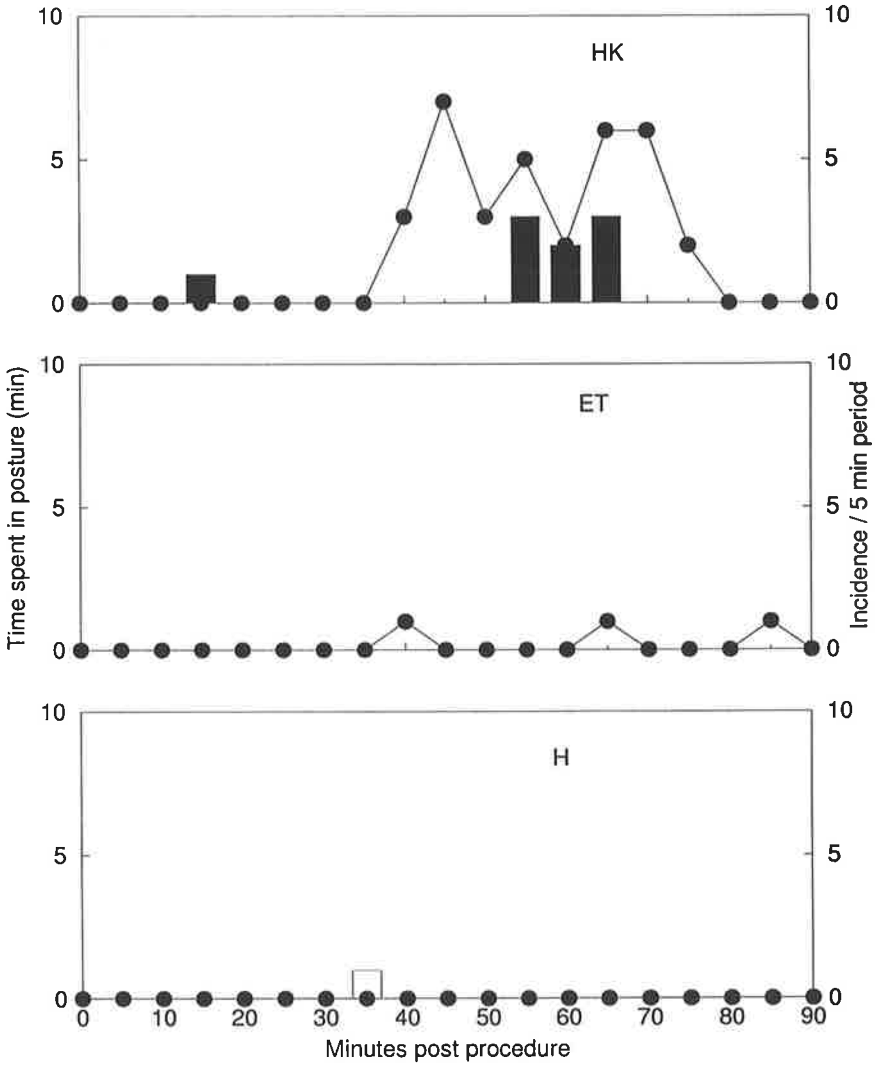


Figure 7.7: Total time spent in abnormal standing/walking (■) and abnormal lying postures (□) and the incidence of RKROLL (•) following control handling (H); ear tagging (ET); tail docking by hot knife (HK) in 6 lambs.

The combination of tail docking and castration by rubber rings produced the largest increase in RKROLL scores and abnormal posture displays of any treatment. Whilst APBs had returned to normal levels by 60 minutes, abnormal lying postures had not returned to normal levels by the end of the 90 minute study period (Figure 7.8). There was also a significant reduction in the amount of time lambs spent standing immobile (Figure 7.6).

7.3.5.3 Mulesing treatments

Lambs in the HKMET treatment group showed no increase in RKROLL behaviours, however, abnormal standing postures were significantly increased in this group and did not return to normal values before the end of the 90 minute study period (Figure 7.9). The total amount of time lambs spent immobile and in statue standing (SS) postures following this treatment group was significantly higher than normal values (Figure 7.6). The pain produced from surgical removal of areas of skin from the breech discouraged any form of lying down, either normal or abnormal, in this group (Table 7.4).

Lambs receiving the same treatment with the addition of rubber ring castration (HKRRCMET) showed a significant increase in active pain behaviour in comparison to handled only (H) and HKMET lambs. There was a significant increase in abnormal posture displays following this treatment that had not diminished by the end of the 90 minute study period (Figure 7.9). The incidence of abnormal standing and lying postures was reasonably evenly distributed (Figure 7.6).

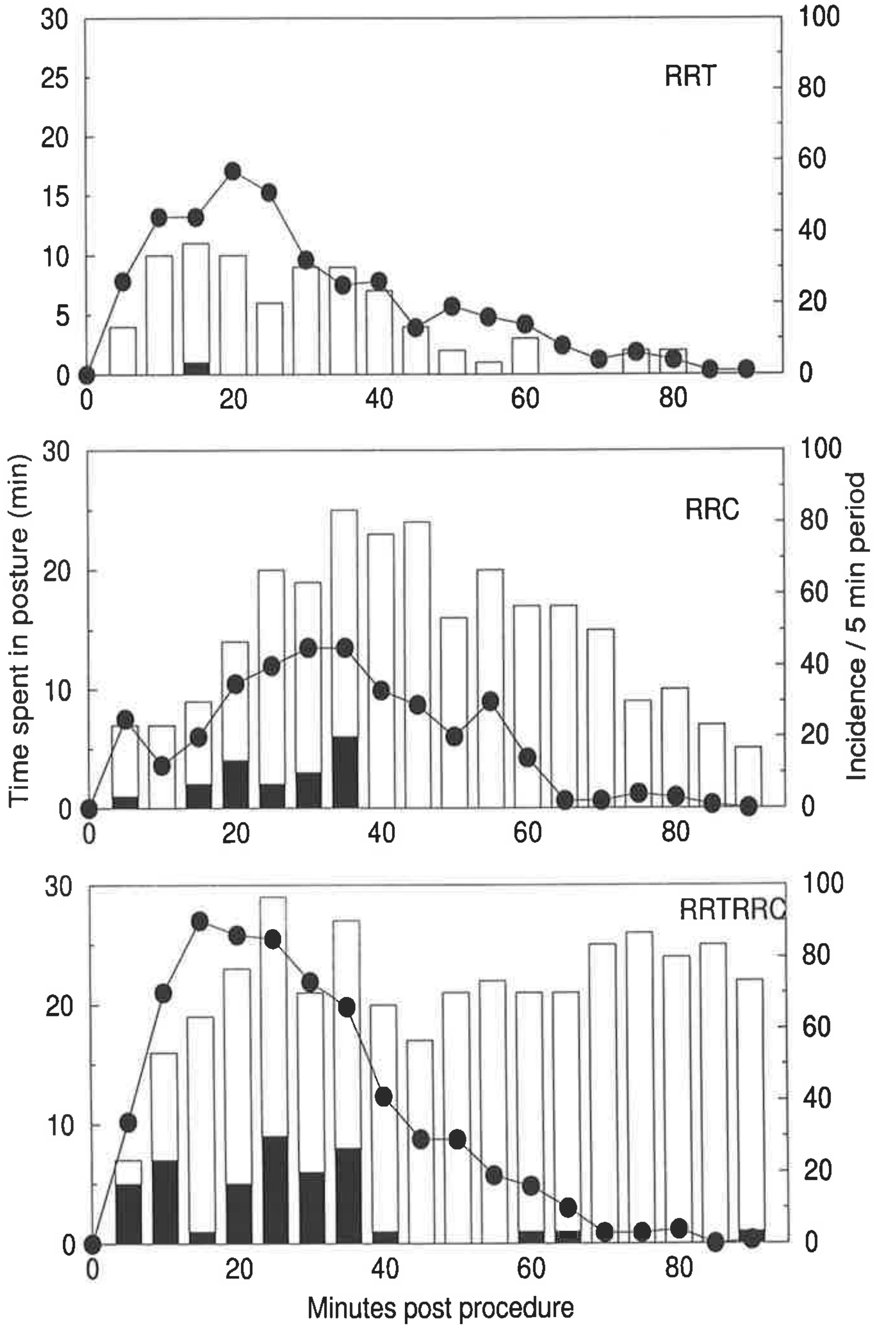


Figure 7.8: Total time spent in abnormal standing/walking (■) and abnormal lying postures (□) and the incidence of RKROLL (•) following tail docking by rubber rings (RRT); castration by rubber rings (RRC); tail docking and castration by rubber rings (RRTRRC) in 6 lambs.

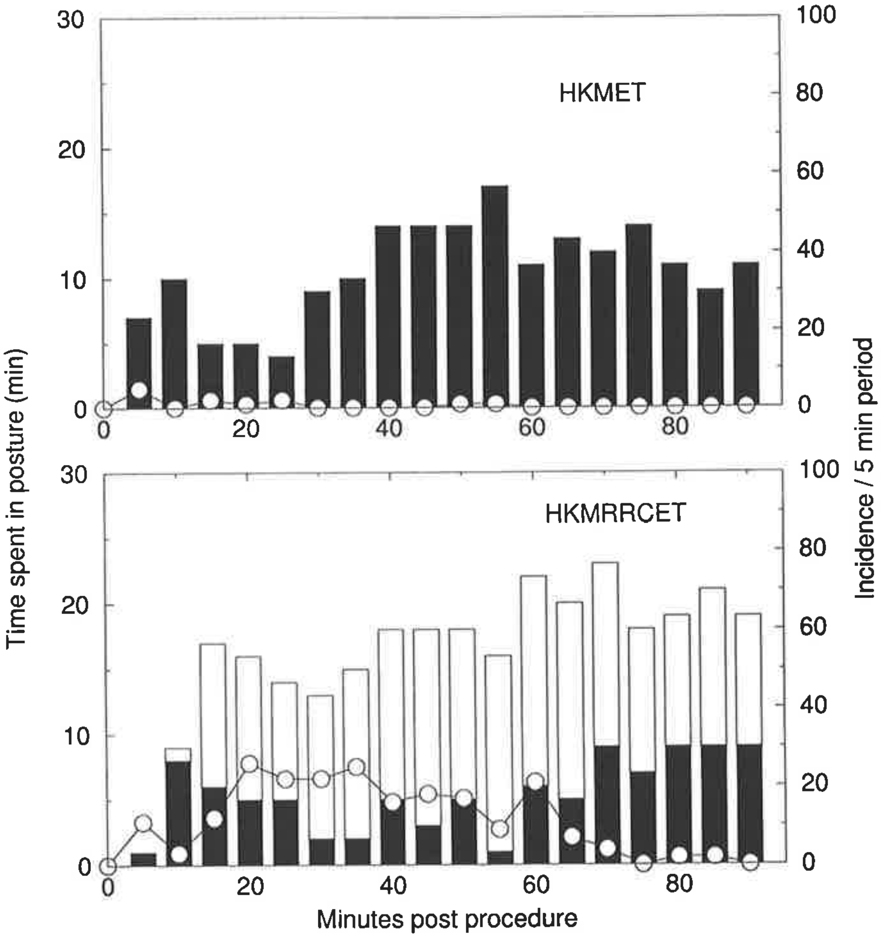


Figure 7.9: Total time spent in abnormal standing/walking (■) and abnormal lying postures (□) and RKROLL (○) following tail docking by hot knife plus Mulesing plus ear tagging (HKMET); tail docking by hot knife plus castration by rubber rings plus Mulesing plus ear tagging (HMRRCMET) in 6 lambs.

7.4 Discussion

The intention of this study was to examine the behavioural and postural responses to the pain of husbandry procedures in lambs to provide a comparison between the husbandry treatments and as validation of the measures used.

7.4.1 Comparison of husbandry treatments

The painfulness of the various treatments correlated with previous findings and presumed painfulness based upon assumptions regarding the amount of tissue involved and the site. The behavioural and postural responses seen in the present study suggest a ranking of H < ET < HK < RRT < HKMET < RRC < HKRRCMET < RRTRRC.

Ear tagging (ET) produced no evidence of pain using the behavioural and postures measures. While, puncturing the pinnae of the ear would be expected to produce some pain, it was not of a degree that produced measurable postural or active pain responses.

Tail docking by hot knife produced an increase in active pain behaviour and abnormal postures, however this response was not statistically different from control lambs. This concurs with previous findings of no significant behavioural or hormonal stress response to tail docking by hot knife (Graham et al., 1997). Tail docking produced the largest response at the time of application of all the procedures examined in this study, with vocalising and struggling being commonly seen at the time of tail docking. This reaction appeared to be related to the amount of time required to fully severe and cauterise the tail. Knife temperatures that were too low produce slow, prolonged cutting times, whilst high knife temperatures produced rapid cutting but inadequate cauterisation of the wound site. The time course of pain following tail docking by hot knife appeared to be delayed and apart from the responses seen at the time of tail docking there appeared to be few indicators of pain initially. Most of the behavioural and postural responses were observed 40 minutes after the treatment. This phenomenon is consistent with the delayed onset of burn pain reported in many human burns patients; proposed causes of this delay are shock and destruction of nerve endings as a result of deep injuries (Choiniere, 1994). Burn pain is a complex and specific pain type - the majority of behavioural and postural measures used in this study have been developed in studies of surgical and ischaemic pain types. It may be that specific measures for burn pain need to be developed to elucidate the pain response from hot knife tail docking.

Tail docking by rubber rings produced large behavioural and postural responses indicative of pain. This pain response was far greater than that of the alternative tail docking method using a hot knife. It appears that the tight rubber rings are sufficient to prevent blood supply to an area but do not prevent the conduction of nerve impulses from the painful, ischaemic tail tissue. Reductions in active pain behaviour can be achieved by crushing the tail distal to the point at which the rubber ring is applied, presumably preventing the conduction of nerve impulses at this point. However, this still does not reduce the incidence of abnormal postures (Graham et al., 1997). This is in contrast with methods such as hot docking iron that remove the entire tail and all the nociceptive receptors contained within leaving only nociceptors in the tail stump to transmit painful stimuli. The choice of tail docking methods in lambs appears to be dependent upon cultural differences, economic factors and perceptions of lamb welfare. Based upon the data presented in this study, tail docking by hot knife appears to offer significant welfare advantages over tail docking by rubber rings. However, proponents of tail docking by rubber rings claim a cleaner, easier method and argue that tail docking by hot knife can lead to significant blood loss and slow wound healing.

The treatments involving castration by rubber rings, (RRC, RRTRRC, HKRRCMET) all resulted in significant increases in the time lambs spent in abnormal lying postures. Whilst active pain behaviours had all returned to normal values by the end of the 90 minute experimental period, the incidence of abnormal postures was still above normal levels. Castration appears more painful than tail docking; active pain behaviours are similar for both treatments however castration produces a far greater number of abnormal posture displays. The combination of rubber ring tailing and rubber ring castration appeared to be the most painful treatment in this study producing the largest behavioural and postural responses.

Mulesing without the ischaemic pain from rubber ring castration (HKMET) produced few active pain behaviours. It has been suggested that Mulesing produces stimulation induced analgesia and the release of endogenous opioids (Fell and Shutt, 1989). This stress induced analgesia mechanism for β-endorphin release appears in response to surgical stimulation and not ischaemic pain from rubber rings (Shutt et al., 1988a). However, the significant increase in abnormal posture displays (Figures 7.4 and 7.9), reduction in total travel (Table 7.3) and the increase in statue standing (Table 7.4) and immobile periods (Figure 7.6) suggest the reduction in active pain behaviours may be a product of attempts by the lamb to minimize stimulation to painful areas.

Mulesing with RRC (HKRRCMET) combined responses from the two different pain types (surgical and ischaemic). In comparison to the handling only (H) and HKMET treatments, there was a significant increase in the time spent in abnormal lying postures and in the incidence of active pain behaviours.

It would appear that the ischaemic pain from rubber ring castration prevented the lamb from adopting strategies to minimize stimulation to the breech area by reducing total movemement, as seen following HKMET. If the β-endorphin release seen following sugical castration and Mulesing (Fell and Shutt, 1989; Shutt et al., 1988a) is a form of endogenous analgesia it appears to be specific to that pain type and ineffective at treating the ischaemic pain from rubber rings. The β-endorphin release from Mulesing provided no reduction in the incidence of active pain behaviour in the HKRRCMET group in comparison to the RRC treatment group. It was not possible to determine if differences in behavioural and postural scores between the HKMET and HKRRCMET treatment groups were due to sex differences. Lambs were assigned to treatment groups as would be the case in a normal farm environment and therefore the HKMET group consisted entirely of female lambs and obviously only male lambs were used in the HKRRCMET treatment group. However, there are no reports of differences in pain response between male and female lambs.

7.4.2 Validation of behavioural measures

The data from this study proves that the use of behavioural and postural indicators to assess pain in lambs following husbandry treatment can be applied to not only the previously proven rubber ring methods but also to surgical procedures such as Mulesing.

Not all of the behavioural indices recorded during this study proved useful. Tail wagging was a poor differentiator between treatment groups and did not rank well with presumed painfulness of procedures. It has been postulated that the perhaps separate recording of frequency and amplitude of individual 'wags' would be more effective (Kent et al., 1995). In the present study tail wagging was more generally associated with teat seeking and suckling activities in lambs; tail wagging episodes were also difficult to observe following complete removal of the tail from hot knife docking. Vocalisation of the lamb also did not correlate well with the severity of the treatment and its expression was highly variable amongst lambs. The overall vocalisation and APB data was capable of being overwhelmed by the incessant vocalisation of a single lamb.

Determining the most effective indices required for an accurate delineation between treatments is important. The ideal pain measure would involve the use of purely objective measures. The most effective combination of indices in this study (AbPos, RKROLL, travel, immobile), involved only indices for which specific criteria for their categorisation had been created. During the behavioural studies in this thesis, this author found difficulty assigning some previously used behavioural and postural classifications with any great certainty. The incidence of abnormal standing postures (S2 and S3) was lower in this study than previous studies by others (Molony et al., 1993; Kent et al., 1998). This may reflect the subjective component of such assessments and the large variation in what may be considered normal behaviour. The classification a lamb is given is often unavoidably based upon the observers knowledge of the treatment the lamb has received. An untreated lamb may exhibit non-pain playful behaviour such as 'gambolling' that when compared to the gaits of other lambs could be classified as abnormal. Similar problems were encountered when assigning the easing quarters posture. Whilst it was an obvious indicator of discomfort, the variation between lambs in the degree to which the hindquarters were lowered and qualitative component involved in assessing the threshold between normal and abnormal states made it, at times, difficult to assign confidently. This subjective component of some behavioural and postural measures can introduce inaccuracy and can lead to a variation in scoring when pain is assessed by different people (Holton et al., 1998).

However, the lying postures were easy to score objectively as they have been comprehensively classified in an almost mechanistic fashion based upon the degree of limb extension. Postural changes, whilst less dramatic perhaps than changes in active pain behaviour were more succesful in describing the pain experience following husbandry treatments. The measurement of abnormal posture displays offered a number of advantages over APBs alone;

1. The relationship between presumed severity and abnormal posture displays was much better than that of APBs.
2. Treatments such as HKMET whilst obviously painful produced no active pain response.
3. Regardless of the treatment, active pain behaviours had returned to normal levels within 70 minutes, postural displays indicative of pain continued much longer.

APBs may adequately describe the acute pain response but may be limited in their duration and scope. This may be due to :

1. The expenditure of energy involved and physical reserves becoming exhausted by such active displays.
2. The conflict between such exhibitions of active pain and stimulation of painful wound sites, as seen in the HKMET treatment group.
3. The predator signal risk. The sheep is a defenceless animal who limit outward behavioural displays that may identify it as a potential target for

predators such as foxes. For this reason behaviours such as flocking and normal grazing activities are maintained by sheep even whilst suffering the most severe afflictions, such as fly strike. Active pain responses would not only limit the ability of sheep to engage in normal flock behaviours but would also present a more obvious cue than the subtler postural changes to a potential predator.

4. Localised vs. general pain. In the current study the poorly localised pain produced by Mulesing generated few APBs, similar effects have been seen when comparing castration by rubber ring and surgical methods (Molony et al., 1993; Thornton and Waterman-Pearson, 1999). APBs may be poor indicators of vague, poorly localised pain.

It was hoped that the use of travel data and the calculation of immobility periods from this data would provide a purely objective index that could be used to help classify pain in lambs. Immobility and travel were useful in assigning lambs to their treatment groups and are easily acquired purely objective indices. Their use as stand alone indices was limited, but immobile and travel values provided qualitative supplemental data for some treatments.

Other possible indices that were observed during the present study and may be of use in future studies of pain in lambs were:

- Walking backwards which was often seen in the higher ranked painful groups. A definition of 4 or more steps backwards may offer a starting point for its categorization.

- Distance between lamb and mother. For studies where it was possible to match lamb and ewe, the position of the mother in the pen was recorded. From this, the distance between the lamb and it's mother can be calculated for each time period. The "following" instinct is strong in young lambs, and fright and distress cause flocking behaviour (Hulet et al., 1975). Increases in the normal distances between mother and lamb would signal alterations in normal behaviour in lambs and in an open field situation could have significant consequences for mis-mothering. During the studies in this thesis it was not always possible to match lamb and mother pairs, however in single studies the data suggests evidence of the usefulness of such measures. The recording of such spatial relationships would be even more useful in a paddock environment where normal flock behaviour would create greater travel distances and create more "following" pressure for lamb and mother.

Ideally an integrated score combining objective measures such as active pain behaviours, posture, immobility and travel could provide a readily determined measure that could be used by flockmasters and researchers to provide better care or understanding of painful treatments. The appropriate weighting for combining scores from different measures with different distributions needs to be discerned. The actual weighting value for each score due to the different range and distribution was beyond the scope of this study but some issues were identified during the course of these studies.

- The relationship between some measures is also unknown, for instance, an increase in immobile periods leads to a reduction in APBs. The relative contribution of each in expressing the total pain experience is unknown.
- Different pain types can cause an alternative expression of the same behaviours, such as the reduction in APB seen in HKMET as opposed to the large increase in APB following RRCRRT.

- Treatments with a combination of pain types offer challenges in assessment, such as when the conflict between reduced activity levels following Mulesing is combined with the increased restlessness from RRC, as in the HKRRCMET groups.

Conclusion

This study provides data on the relative painfulness of some standard husbandry procedures that are performed on lambs as a part of normal flock management. The recording of active pain behaviours and abnormal posture displays offers a method of assessing and comparing the painfulness of treatments that generate differing pain types. The ranking of the husbandry treatments provides an indication of the relative painfulness of such treatments and may offer objective assistance when assessing the merits of alternative procedures such as tail docking by hot knife or rubber rings.

Many of the husbandry treatments examined in this chapter are obviously painful, the use of alternative procedures or the application of pharmacotherapy for existing procedures may provide improvements in the welfare of lambs undergoing painful husbandry procedures. This chapter provides both evidence for the need for such improvements, and a baseline from which to measure such improvements. The usefulness of xylazine in providing pain relief following painful husbandry procedures will be examined in the next chapter.

Chapter 8

The influence of intramuscular xylazine on the behavioural responses of lambs to painful husbandry procedures

8.1 Introduction

Castration, tail docking and Mulesing of young lambs are routine procedures on many sheep farms, and are performed in the interests of disease prevention, higher productivity and to prevent indiscriminant breeding. There are a number of alternative methods for performing most of these procedures including surgery, tight rubber rings, hot docking iron and burdizzo. The choice of husbandry treatments and methods can vary between farms and countries based upon perceptions of lamb welfare, economic considerations, the technical skill available and conditions particular to that area.

Australian flockmasters favour economic husbandry procedures due to the generally large flock sizes. Australian merino sheep are also susceptible to blowfly strike, that is, oviposition in damp wool by *Lucilla cuprina* followed by invasion of the skin by larvae. To protect against flystrike, the modified Mules operation is performed on lambs; this involves surgical removal of wool and skin from the breech area. Linear scars form over the wound which stretch the skin surrounding the anal area leaving an area free of wool that greatly reduces the risk of flystrike (Watts et al., 1979).

In other countries such as the UK, environmental and sheep breed differences mean flystrike is less prevalent and the Mulesing operation is usually not performed. In recent decades, surgical methods of castration and tail docking have largely been replaced by the use of tight rubber rings.

Concern regarding the pain and suffering from such procedures has produced debate over the welfare of lambs (Anonymous, 2001; Stevenson, 1994) and in the UK, guidelines regarding the performance of such procedures (Anonymous, 1994). Attempts have been made to develop alternative husbandry procedures or to reduce the pain from existing procedures. Spinal administration of local anaesthetics has been shown to reduce the pain from surgical castration (Scott et al., 1996) and rubber ring tail docking procedures (Wood et al., 1991; Graham et al., 1997). Also, infiltration of the site with local anesthetics can reduce the pain of castration (Kent et al., 1998; Thornton and Waterman-Pearson, 1999) and tail docking by rubber rings (Graham et al., 1997). However, local anaesthetics have not been adopted for widespread use due to the technical difficulties of epidural and intrathecal injections, or the multiple injections required to ensure adequate anaesthesia of an area when infiltration is used. Diclofenac, a non-steroidal anti inflammatory drug (NSAID) has been shown to reduce the pain response from combined rubber ring/burdizzo castration (Molony et al., 1997) but not the pain of tail docking by rubber rings alone (Graham et al., 1997).

Alternative mulesing methods have included depilation of the breech by high energy beam (Sorell et al., 1990) and the application of a quartenary ammonium compound (Chapman et al., 1994). All alternative Mulesing procedures have proved either impractical or offered no welfare benefit over standard surgical procedures. There have been no studies examining the use of analgesics for the reduction of pain from Mulesing.

Previous chapters showed that the α_2-agonist xylazine had significant antinociceptive effects in sheep and lambs when administered intramuscularly (Chapters 3, 5 and 6) to treat experimentally induced pain. The use of xylazine during painful husbandry procedures could provide substantial welfare benefits to millions of lambs each year. However, the relationship between the reduction of acute experimentally induced pain from electrical stimulus and inferences to the longer term pain from painful husbandry procedures is not known.

The aim of this chapter was to examine if intramuscular injection of the α_2-agonist xylazine could reduce the behavioural and postural indicators of pain seen following a range of commonly used husbandry procedures.

8.2 Methods

8.2.1 Animal preparation

All studies were performed at the Institute of Medical and Veterinary Sciences, Windarra Farm, Farrell Flat, South Australia. Lambs aged 4–6 weeks and a mean weight of 11.1 ± 0.5 kg. were paired with their mothers and placed in yards as described in Section 7.2.3 of Chapter 7.

8.2.2 Study design

8.2.2.1 General design

A stock xylazine solution of 0.5 mg/ml was prepared using 0.9% saline to enable easy dosing calculations, whereby a 50 μg/kg dose equated to 1 ml per 10kg of lamb bodyweight. Lambs were administered 50 μg/kg of xylazine IM (Troy Laboratories, Smithfield NSW, Australia) 15 minutes prior to the commencement of husbandry procedures. This interval ensured peak analgesic effect during the procedures, and was based upon anti-nociception data from Chapter 3. Unlike studies in adult sheep (Chapters 3, 4 and 5), dosing was adjusted per kg of body weight because of possible variation in weight between lambs due to their variable growth rate at this age. Following drug administration lambs were returned to their mothers until the time of husbandry procedures. Groups of six lambs received one of the following treatments:

- tail docking by rubber ring (RRTxyl)
- tail docking by rubber ring + castration by rubber ring (RRTRRCxyl)
- tail docking by hot knife + Mulesing + ear tagging (HKMETxyl)
- tail docking by hot knife + Mulesing + castration by rubber ring + ear tagging (HKRRCMETxyl)

The treatments were based upon the combinations of husbandry procedures commonly performed on lambs in normal farm environments in Australia. Each of the husbandry procedures is fully described in Section 7.2.3.2 of Chapter 7.

8.2.2.2 Behavioural measurements

Video tapes of each study were reviewed at a later date, behaviours and postures were recorded using the behavioural software described in Section 7.2.2 of Chapter 7.

8.2.3 Data analysis

The husbandry treatments from this study using xylazine were compared to similar unmedicated husbandry treatments from Chapter 7. Differences in behavioural and postural indicators between medicated and unmedicated treatments were assessed using the Mann-Whitney test. A P value of less than 0.05 was assumed to be significant.

8.3 Results

The general findings were that most xylazine treatment groups showed an increase in travel distance over the experimental period (Figure 8.1) and a reduction in immobile periods (Figure 8.2), although these values were statistically significant in only one of the treatment groups. There was no evidence of sedation or respiratory depression as a result of xylazine administration in any of the treatment groups. Furthermore, there were no observations of hyperventilation or panting in these lambs. The effects of xyalzine on specific husbandry treatments are discussed in detail below.

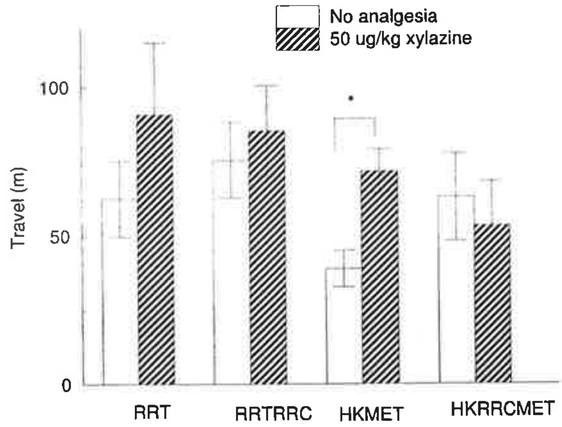


Figure 8.1: Total distance travelled during the 90 minute experimental following, tail docking by rubber ring (RRT); tail docking and castration by rubber ring (RRTRRC); tail docking by hot knife + Mulesing + ear tagging (HKMET); tail docking by hot knife + Mulesing + castration by rubber ring + ear tagging (HKRRCMET), with and without 50 μg/kg xylazine IM. (Mean ± SEM) of 6 lambs. * indicates $P < 0.05$.

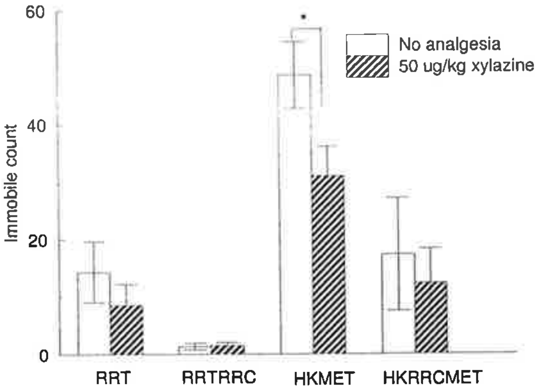


Figure 8.2: Number of periods lambs were immobile for greater than 1 minute following, tail docking by rubber rings (RRT); tail docking and castration by rubber rings (RRTRRC); tail docking by hot knife + Mulesing + ear tagging (HKMET); tail docking by hot knife + castration by rubber rings + Mulesing + ear tagging (HKRRCMET), with and without 50 μg/kg xylazine in 6 lambs (Mean ± SEM). * indicates $P < 0.05$.

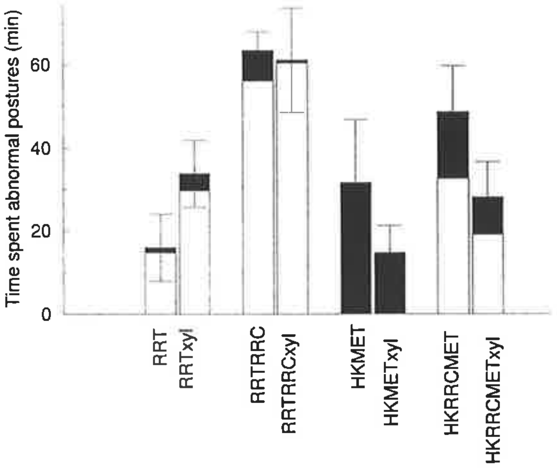


Figure 8.3: Time spent in abnormal postures following, tail docking by rubber ring (RRT/RRTxyl); tail docking and castration by rubber ring (RRTRRC/RRTRRCxyl); tail docking by hot knife + Mulesing + ear tagging (HKMET/HKMETxyl); tail docking by hot knife + Mulesing + castration by rubber ring + ear tagging (HKRRCMET/HKRRCMETxyl), without and with 50 μg/kg IM xylazine in 6 lambs. (Mean ± SEM). Abnormal standing/walking (☐), abnormal lying (■).

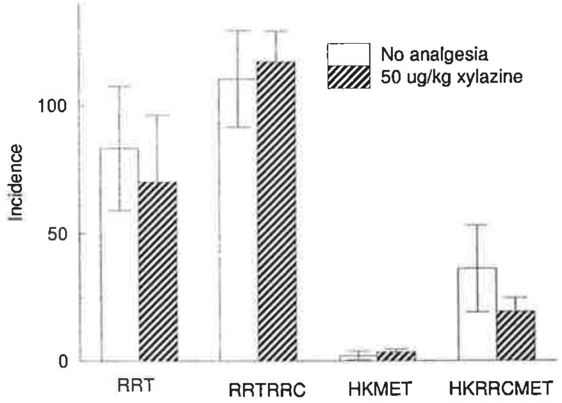


Figure 8.4: The incidence of active pain behaviours (RKROLL) following tail docking by rubber ring (RRT); tail docking and castration by rubber ring (RRTRRC); tail docking by hot knife + Mulesing + ear tagging; tail docking by hot knife + castration + Mulesing + ear tagging, with and without 50 μg/kg xylazine in 6 lambs.

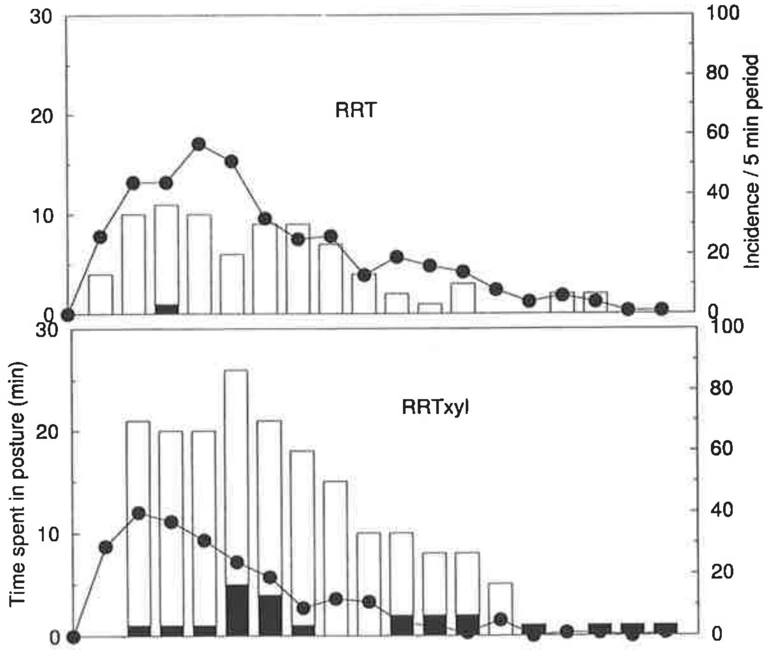


Figure 8.5: Total time spent in abnormal standing/walking (■), abnormal lying postures (□) and the incidence of RKROLL (•) following tail docking by rubber rings, with (RRTxyl) and without (RRT) 50 μg/kg of xylazine IM in 6 lambs.

8.3.1 Tail docking by rubber rings (RRT)

Application of xylazine to lambs tail docked using rubber rings slightly reduced the number of immobile periods (Figure 8.2) and increased the total distance travelled during the course of the study (Figure 8.1). The incidence of active pain behaviours (RKROLL) was slightly reduced (Figures 8.4 and 8.5) and the total time spent in abnormal postures, particularly abnormal lying, was increased (Figures 8.3 and 8.5). None of these changes however were statistically significant.

8.3.2 Tail docking and castration by rubber rings (RRTRRC)

The administration of xylazine prior to tail docking and castration by rubber rings produced no statistically significant changes in immobile periods,

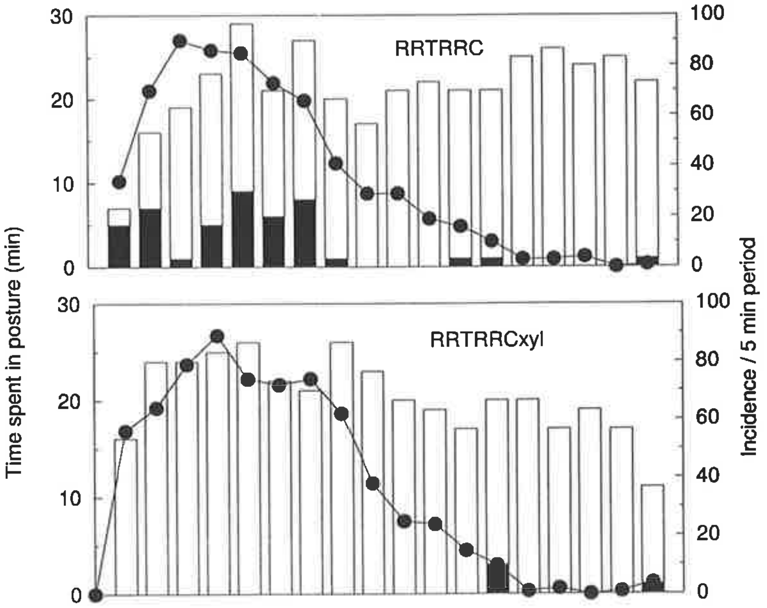


Figure 8.6: Total time spent in abnormal standing/walking (■), abnormal lying postures (□) and the incidence of RKROLL (•) following tail docking and castration by rubber rings, with (RRTRRCxyl) and without (RRTRRC) 50 μg/kg of xylazine IM in 6 lambs.

abnormal postures, travel distance or RKROLL scores in comparison to untreated lambs (Figures 8.2, 8.3, 8.1, 8.4 and 8.6).

8.3.3 Tail docking by hot knife + Mulesing + ear tagging (HKMET)

Lambs administered xylazine showed no changes in RKROLL scores (Figure 8.4), there was a reduction in time spent in abnormal postures from 31.67 ± 15.2 to 14.83 ± 6.39 minutes (Figure 8.3 and Figure 8.7), this reduction was not statistically significant. There was a significant reduction in immobile periods (Figure 8.2) and an increase in travel distance during the experimental period (Figure 8.1).

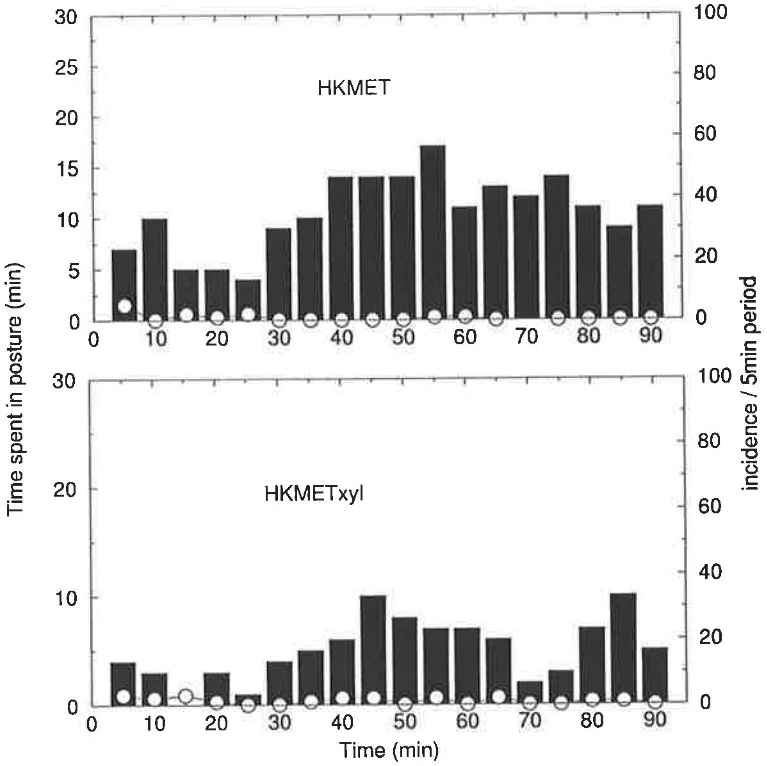


Figure 8.7: Total time spent in abnormal standing/walking (■), abnormal lying postures. (□) and RKROLL (○) following tail docking by hot knife plus Mulesing plus ear tagging, with (HKMET) and without (HKMETxyl) 50 μg/kg xylazine IM in 6 lambs.

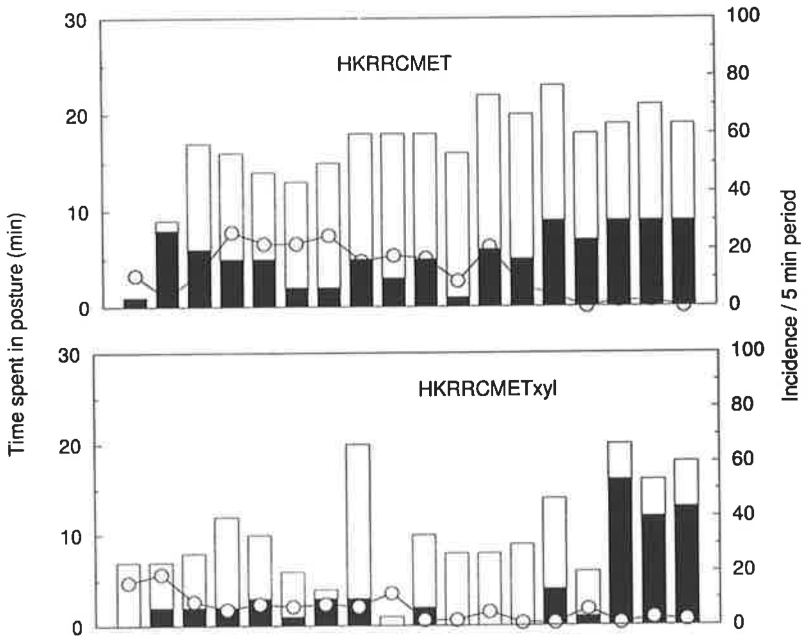


Figure 8.8: Total time spent in abnormal standing/walking (■), abnormal lying postures. (□) and RKROLL (○) following tail docking by hot knife plus castration by rubber ring plus Mulesing plus ear tagging, with (HKRRCMET) and without (HKRRCMETxyl) 50 μg/kg xylazine IM in 6 lambs.

8.3.4 Tail docking by hot knife + castration by rubber rings + Mulesing + ear tagging (HKRRCMET)

Lambs that received tail docking by hot knife plus castration by rubber rings plus Mulesing plus ear tagging showed a reduction in abnormal postures (Figures 8.3 and 8.8), RKROLL scores (Figures 8.4 and 8.8), immobile periods (Figure 8.2) and total travel distance (Figure 8.1) when treated with xylazine. However, none of these changes were statistically significant.

8.4 Discussion

The data from this study suggests that analgesic therapy using intramuscular xylazine is more useful in reducing the indicators of pain from husbandry treatments involving surgical procedures, such as Mulesing and hot knife tail docking, rather than treatments that use rubber rings.

8.4.1 Rubber ring treatments

Xylazine appeared ineffective at reducing the behavioural indicators of pain resulting from tail docking and/or castration using rubber rings. In fact, for lambs tail docked using rubber rings, xylazine appeared to increase the time spent in abnormal postures. This is similar to increases in active pain behaviour seen in rubber ring tail docked lambs treated using the non-steroidal anti-inflammatory, diclofenac. This anamoly was attributed to individual variations in the response of lambs. Three lambs in the treatment group not receiving diclofenac were classified as 'low responders' in that they showed a limited response to rubber ring tail docking, whilst only one of the diclofenac treated lambs showed a limited response (Graham et al., 1997). This variation in response was also apparent during the behavioural studies in this thesis and may have been a factor in some obvious behavioural differences between treatments failing to reach statistical significance. The confounding increase in a pain behaviour measure following analgesic therapy, can also be explained if abnormal postures and active pain behaviours are considered alternative expressions of the same responses. In the present study, compared to unmedicated RRT lambs, lambs in the RRTxyl group showed increases in abnormal postures but also a decrease in active pain behaviours (RKROLL). In the diclofenac study (Graham et al., 1997), lambs tail docked by rubber ring and receiving diclofenac showed an increase in active pain behaviour, but also reductions in abnormal postures in comparison to lambs not administered diclofenac. As discussed in Section 7.4.2 of Chapter 7, it is important to assess the relative contribution of each measure to the total pain experience. There have been no reports of hyperalgesic effects associated with xylazine administration (Nolan et al., 1987a; Grant et al., 1996).

Local anaesthetics have proven effective at reducing some of the pain response to rubber ring castration and tail docking (Graham et al., 1997; Molony et al., 1997), however, the 'visceral' testicular pain produced by rubber ring castration has been described as resistant to alleviation by commonly used analgesics (Molony and Kent, 1997). It appears that xylazine may be another analgesic that is ineffective at treating such forms of pain.

8.4.2 Mulesing treatments

Important behavioural and postural indicators of pain were reduced by the application of xylazine prior to the HKMET treatment. This is a common procedure for ewe lambs on Australian wool producing properties. The corresponding procedure for male lambs (HKRRCMET) is identical but involves castration, usually by rubber ring. Xylazine was less successful in reducing the pain response to this procedure. Not only were abnormal postures reduced in HKMET lambs but time spent immobile was reduced and perhaps consequently total travel distance increased. This increase in mobility seen as a result of xylazine administration may not only signal a positive outcome in terms of pain relief but could also reduce the risk of mis-mothering in the larger flock environment. The reluctance of the lamb to move can cause stress for the ewe and lamb due to the strong flocking instinct in sheep. A weak lamb/dam bond may cause the lamb to be abandoned by the ewe in favour of the larger flock, or lamb and dam may lag behind and remain away from the larger flock. This pressure is increased in larger paddocks where flocks travel greater distances creep feeding and to find water. Separation of an unweaned lamb from its mother is obviously disastrous, but either scenario creates an increased risk of predation from animals such as foxes, when sheep are distanced from the safety of the larger flock.

8.4.3 Conclusions

The results from this study were encouraging but not definitive. Xylazine appeared to reduce the pain of surgical husbandry procedures but not of a sufficient degree or duration to always show a statistically significant effect. Fortunately, dose and preparation manipulations can provide altered pharmacokinetic/dynamic responses or alternative α_2-agonists may provide greater, longer lasting analgesia (Muge et al., 1994; Tranquilli and Maze, 1993). Due to many standard analgesics being ineffective in sheep (Nolan et al., 1987b; Grant et al., 1996) the confirmation of a suitable agent that can reduce some of the behavioural responses to the pain of husbandry procedures is a major step forward. Further studies examining alternative agents and dosing strategies could further reduce the pain from such procedures.

For husbandry treatments involving the application of tight rubber rings xylazine offered no pain relief. For these procedures alternative analgesics need to be investigated. The effectiveness of a non steroidal anti-inflammatory agent such as diclofenac in treating the pain from tail docking using rubber rings is uncertain (Graham et al., 1997). However, diclofenac in combination with the use of burdizzo appears effective in treating the crushing pain from burdizzo application during castration (Molony et al., 1997). Many standard husbandry treatments combine a number of different pain types, such as the HKRRCMET treatment group in this chapter, no suitable single analgesic has yet been identified that is capable of treating such forms of multi-modal pain. Identifying suitable analgesics for each pain component could provide a basis for developing an analgesic cocktail, analgous to neuroleptanalgesics that combine a neuroleptic agent with an opioid to provide both sedation and analgesia during minor procedures in animals (Corssen et al., 1964).

An alternative to 'pick and mix' analgesic and husbandry combinations may be to select husbandry procedures which are proven to be amenable to treatment by analgesics. Surgical methods of tail docking and castration have largely been replaced by the use of rubber rings due to factors of convenience and the reduced risk of infection and blood loss (Filmer, 1938; Ewer, 1942), despite surgical and hot docking iron methods appearing to be less painful (Molony et al., 1993; Thornton and Waterman-Pearson, 1999). If an analgesic such as xylazine is shown to effectively treat the surgical pain, these methods could be reconsidered or refined, and when used in conjunction with an analgesic may provide substantial welfare benefits for lambs. This chapter provides evidence that xylazine can effectively treat some of the pain components from surgical husbandry procedures and creates a focus for further investigation into the use of α_2-agonists during surgical husbandry procedures.

Chapter 9

General discussion, conclusions and future directions.

The welfare of animals is an increasingly topical and emotive issue. Great progress has been made to improve the welfare of animals used in farming industries and biomedical research, and legislation in many countries ensures the humane treatment of most animal species. The pharmacological management of pain in domestic animals is as familiar to us as its clinical application in humans. However, these forms of pain management are generally applied to our companion animal species, such as dogs and cats, with whom we have formed emotive/sympathetic bonds. Within the farming environment millions of lambs each year are subjected to painful husbandry procedures such as tail docking, castration and Mulesing. Whilst recommendations and even legislation in some countries provide guidelines on the timing and choice of procedures employed, the use of analgesics to treat the pain from such procedures is limited. There are two major reasons for the failure to recognise the need for analgesic pain relief measures in sheep, or the failure to apply them.

1. The painfulness of many procedures was probably under-estimated due to the limited range of behavioural pain displays in sheep. Whilst most of us are familiar with the indicators of pain in domestic pets, such as dogs and cats, we can fail to recognise the more subtle indicators displayed by sheep in pain. The recent refinement of methodologies to assess the objective behavioural indicators of pain have identified the considerable pain following tail docking and castration by tight rubber rings and surgical methods. The important behavioural changes seen in response to such pain can be as subtle as foot stamping and postural changes reflected as the degree of leg flexion. Whilst the more obvious displays such as vocalising, which are important indicators in other species, are poor descriptors of the pain experience in lambs.

2. The use of analgesics for the treatment of pain in sheep has also been limited due to species specific differences in the action of many analgesics. Algesimetry testing has revealed that many analgesics commonly used to treat pain in animals and humans are ineffective in sheep. Xylazine has shown analgesic properties in sheep but the therapeutic dose range is small and evidence of hypotension, sedation and hypoxaemia have limited further investigation and use. Local anaesthetics have been used experimentally to treat the localised pain from tail docking and castration with some success, but the difficulties of application and treating less localised sources of pain have probably prevented widespread adoption.

The need for further investigation into the identification of suitable analgesics for use in sheep is apparent. The aim of this thesis was to define the safety of low dose xylazine in sheep and lambs using algesimetry and behavioural methodologies. The findings of this thesis are summarised below.

9.1 General discussion of experimental studies

9.1.1 Chapter 3. A comparison of the anti-nociceptive effects of xylazine via three different administration routes

Differences in the degree of anti-nociception produced by identical doses of xylazine due to different administration routes were examined in this chapter using an algesimetry model based on a nociceptive response to a painful electrical stimulus. The degree, duration and time course of analgesic effects were compared between intravenous, intramuscular and subcutaneous administration routes. Intravenous administration provided rapid analgesia, however the duration was short and sedative effects were seen in a number of animals, whilst subcutaneous administration showed a relatively long duration of effect but the onset was slow. These studies suggested that intramuscular administration produced the longest and greatest total anti-nociceptive response of all the administration routes for this dose. Due to its simple application and efficacy, this makes it ideal for routine pain relief in sheep, and it was considered to provide the best balance between onset, duration and magnitude of effect for the studies in this thesis. However, the duration of analgesia provided by a single low dose bolus of xylazine by the intramuscular route may not be sufficient for the treatment of many types of pain, and alternative formulations or repeat dosing may need to be examined.

9.1.2 Chapter 4. The cardiovascular and respiratory effects of low dose intramuscular xylazine in conscious sheep

Published studies have shown that low doses of intravenous xylazine in sheep can produce deleterious cardiovascular and respiratory effects, most notably hypoxaemia. The cardiovascular and respiratory effects of intramuscularly administered xylazine were examined using a catheterised sheep preparation, in which cardiac output, arterial blood pressure, heart rate and arterial blood gases were recorded. No major detrimental side effects which could compromise the well-being of the sheep were found. These findings suggest that one of the major concerns involving the routine use of intramuscular xylazine as an analgesic can be allayed. That is, that the significant cardiovascular and hypoxaemic effects seen following the intravenous administration of xylazine was not produced by intramuscular administration. This is presumably related to the lower peak blood concentration expected for the intramuscular compared to the intravenous route.

9.1.3 Chapter 5. Steady-state analgesia in sheep by continuous xylazine infusion

The aim of this chapter was to validate a practical regimen to provide steady-state analgesia in sheep following surgical procedures, based upon the findings of the previous chapters was validated. Algesimetry testing using a painful electrical stimulus showed that the concept of a single intramuscular loading dose followed by continuous intravenous infusion of xylazine could provide significant steady-state analgesia in adult sheep. Outcomes from this study are gratifying, with the developed protocol currently being implemented for the post-operative care of sheep following orthopaedic surgery within the Institute of Medical and Veterinary Science, Adelaide, South Australia. Sheep are given the intrammuscular bolus loading dose prior to recovery from anaesthesia and a small clockwork pump is attached to the wool to provide a continuous intravenous inusion of xylazine. Anecdotal evidence provided by animal carers and researchers is encouraging, with faster recovery times to normal weight bearing postures and sheep displaying less visible signs of discomfort such as grinding of teeth and loss of appetite.

9.1.4 Chapter 6. The anti-nociceptive efficacy of low dose intramuscular xylazine in lambs

Lambs, and not adult sheep, represent the largest target group for the use of analgesic doses of xylazine. The relief of pain following husbandry procedures in lambs could provide tremendous welfare benefits for millions of lambs each year.

However, assumptions concerning the analgesic efficacy of xylazine have been based upon algesimetry studies in adult sheep, and there had been no algesimetric testing of xylazine in lambs. This chapter compared the anti-nociceptive response of lambs to previously published data from adult sheep using an identical intramuscular dose regimen but with the dose adjusted per kg body weight. No differences in the duration or magnitude of analgesic effects were seen between lambs and adult sheep. This study confirms the efficacy of this scaled dose of xylazine in lambs and discounts possible differences in the analgesic effect between adult sheep and lambs due to developmental factors. An unexpected finding of the study was the difference in adaptability between adult sheep and lambs to the algesimetry testing regimen. Lambs proved much better candidates for the nociceptive testing, learning the behavioural leg lifting response much quicker and more consistently than their adult counterparts.

9.1.5 Chapter 7. Behavioural responses of lambs to common painful husbandry procedures

The ability of behavioural and postural indicators of pain to describe the painfulness of a range of different husbandry procedures in lambs was examined in this chapter. The behavioural and postural indicators recorded were able to rank the painfulness of different husbandry procedures in an order which correlated well with the presumed painfulness based upon independent criteria. This study showed that the postural indicators of pain persist much longer than the active pain behaviours and that the recording of locomotion and travel within the pen area offered another objective measure which was useful in describing the pain experience in lambs. It was important to provide a benchmark and commonality with previous behavioural studies of pain in lambs and determine if the methodolgies used in these other studies were effective in assessing the pain from husbandry procedures, such as Mulesing, to which they had not yet been applied. With the data from this study it was also possible to establish a ranking of the painfulness between different treatments which could aid decision making when choosing between alternative husbandry methods, such as tail docking by hot docking iron or rubber ring.

9.1.6 Chapter 8. The influence of intramuscular xylazine on the behavioural responses of lambs to painful husbandry procedures

This chapter examined if the analgesic efficacy of xylazine, seen in the laboratory and reported in Chapters 3 and 6, could also be achieved in lambs undergoing the husbandry treatments evaluated in Chapter 7. The behavioural and postural indicators of pain recorded in this chapter suggested that xylazine was more effective in treating the surgical pain from Mulesing, rather than the ischaemic pain from the application of tight rubber rings. Reductions in abnormal posture displays were seen in the two different Mulesing treatment groups following the administration of xylazine. Further studies are needed before unequivocal recommendations for pain control can be made as statistical comparisons between some treatment groups suffered from the relatively small sample size (n=6). However, the indentification of an agent which was capable of reducing the pain from a commonly performed (in Australia) husbandry procedure such as Mulesing is an important step towards the goal of pain management in lambs.

9.2 Implications of experimental studies and future directions

The findings in this thesis provide evidence of the suitability of xylazine for the treatment of pain in sheep and lambs. However, they also highlight differences in the ability of xylazine to treat different forms of pain produced by different husbandry procedures. Surgical husbandry methods appear the most amenable to treatment by xylazine. However, behavioural and postural indicators suggest the duration of pain following a procedure such as Mulesing is longer than the duration of analgesic efficacy of a single intramuscular dose of xylazine. Investigation of alternative α_2 agonists may provide better matching of analgesic effect to time course of pain, although the newer α_2 agonists appear to offer greater potency, but with little difference in duration of action. The development of alternative xylazine preparations in a slow release formulation may be more successful and is proposed for future studies.

Methods of castration and tail docking that use tight rubber rings to cause ischaemia are widely employed throughout the wool industry and appear resistant to analgesic therapy using xylazine. Experimentally, only the use of local anaesthetics to obliterate the transmission of painful nerve impulses has proven successful in reducing the pain response to these procedures. The techniques used in these studies were relatively complex requiring multiple injections or spinal administration to achieve consistent effects. Such complexity requires technical skill and reduces the probability that analgesic therapy by such means will ever be widely adopted. The resolution of pain from these procedures is dependent upon a greater understanding of the causes of ischaemic pain and further investigation of suitable agents to reduce that pain. Data from this thesis showed that tail docking by hot knife produced less pain response than the use of rubber rings for the same procedure. Further studies may identify alternatives to castration and tail docking procedures by rubber ring, which are less painful or more amenable to systemic analgesic therapy.

Understanding the behavioural indicators of pain in lambs is not only useful for researchers who can apply these techniques to models of pain research, but could also be useful for the clinical assessment of pain by veterinarians and flock masters. Before this can happen, further objective measures of pain need to be validated and the key behavioural and postural markers of pain identified to simplify the collection of such data. Whilst the behavioural measures of pain used in this thesis effectively described the pain response from a range of different husbandry procedures, it is also apparent that the relationship between pain and the frequency with which these indicators is seen is not linear, nor are all indicators equally expressed in response to a range of different pain types.

The development of an integrated score which asssess the relative contribution of each component with respect to its frequency of expression in normal behaviour, could provide a simple objective measure of pain and the degree to which a particular animal is displaced from normal behaviour. This could be used by flockmasters or veterinarians to identify animals which require some form of intervention or assist veterinarians when considering whether to implement analgesic therapy.

The challenge for researchers is not just in establishing guidelines that can effectively assess pain and to determine methods of reducing this pain, but also in providing a means of disseminating this information to a wider target audience. Despite continuing research and long term anecdotal evidence, the management of pain in sheep and many other species is often inappropriate or non-existent. Further education is required to close the gap between the current understanding of pain management in many animal species within the research environment, and those on the 'shop floor' who should be applying the findings. A large population of animal users are not able to stay abreast of the current insights regarding pain management which appear in the scientific literature. One method of informing a wider audience would be if the merits of these studies could be assessed by relevant experts and added to a database of knowledge regarding the efficacy of specific anaesthetic and analgesic agents in each species, creating a comparative analgesia compendium using evidence based guidelines. This would be similar to guidelines set out by the NHMRC for the management of pain in humans (National Health and Medical Research Council, 1998) and could provide dosage, warnings and other information specific to a single drug in a single species.

Currently there are a number of web sites that offer recommendations and guidelines regarding analgesia and anaesthesia for animals. However, these fall in to the category of being either abstracted forms of information from existing text books or are limited to just a few species mostly the dog, cat and horse. As they are static pages of text it can also be difficult to add or update information. The viability of developing web based guidelines which dynamically serve their content from information in a database is currently being investigated (http://www.adelaide.edu.au/CADB). Such a site could offer access to, and feedback from, a wide range of participants, regardless of location or level of involvement within a formal research environment.

Chapter 10

Appendix

10.1 Behavioural recording software listing

Listed below is the Python code for the behavioural recording software described in Chapter 7. This software requires Python 1.5.2 (http://www.python.org) and the Tkinter (tcl/tk) extension.

10.1.1 Main program module

10.1.1.1 behave.py

Listing 10.1: Main program module

```
#! / usr/bin/env python
#
#
#  Record data from single sheep every minute or so,
# Need to be able to calculate time lying , stnding , vocalising
# distance travelled , number of up and down events


from Tkinter import *
import string , re
import datameth, datawin, widget
from FileDialog import *
from Dialog import Dialog
import yardmap, utils , htmlprint
```

```
class Recorder(Frame):

    def __init__( self , parent = None):
        Frame.__init__(self,parent)
        self.pack(expand=YES,fill=BOTH)
        self.createWidgets()
        self.master.title ("Behavioural␣Recorder")
        self.recumbency=StringVar()
        self.sheepid=StringVar();self.observervar=StringVar()
        self.dateentry=StringVar()
        self.eartagvar=StringVar()
        self.taildockvar=StringVar()
        self.castratevar=StringVar (); self.sex=StringVar()
        self.analgesicvar=StringVar(); self.mulesingvar=StringVar()
        self.analgdosevar=StringVar();self.analgadmvar=StringVar()
        self.maxtimevar=IntVar(); self.clockstartvar=StringVar()
        self.timeincrvar=IntVar()
        self.realwidthvar=DoubleVar();self.realheightvar=DoubleVar()

        #some initial values
        self.clockstart='0:0'
        self.timeincr =1 # time increment ==1 minute
        self.newx=0 # to make sure no error from map
        self.newy=0
        self.detailscomments=''
        self.alarmonvar=IntVar()
        self.alarmonvar.set(1)
        self.tk_focusFollowsMouse()

    def createWidgets(self):
        self.makeMenubar()
        self.makeMainframe()
        self.makeButtonBar()

    def makeMenubar(self):
        self.menubar = Frame(self,relief = RAISED, bd=2)
        self.menubar.pack(side=TOP, fill=X)
        self.menus()

    def makeIconbar(self):
        """make␣an␣icon␣bar,␣maybe...alarm␣on/off"""
        #self.iconbar=Frame(self,relief = RAISED, bd=2)

    def menus(self):
        File_button = Menubutton(self.menubar,text = "File" , underline = 0)
        File_button.pack(side=LEFT)
        File_button.menu = Menu(File_button)
        File_button.menu.add_command(label='New␣Experiment', underline=0,command=self.newfile)
        File_button.menu.add_command(label='Open␣existing␣file',
                command=self.openfile)#file select dialog
        File_button.menu.add_command(label='Print␣Data␣to␣html␣file', underline=0,
                command=self.printhtdata)
        File_button.menu.add_command(label='Transfer␣file␣data␣to␣database')
```

```
        File_button['menu'] = File_button.menu

        Help_button = Menubutton(self.menubar,text = "Help", underline = 0)
        Help_button.pack(side=RIGHT)
        Help_button.menu = Menu(Help_button)
        Help_button.menu.add_command(label="About")
        Help_button['menu'] =Help_button.menu

        Options_button=Menubutton(self.menubar,text = "Options", underline = 0)
        Options_button.pack(side=RIGHT)
        Options_button.menu = Menu(Options_button)
        Options_button.menu.add_command(label="Change␣Experimental␣Details", command=self.table_alter)
        Options_button.menu.add_command(label="Display␣Setup", command=self.setup)
        Options_button['menu'] = Options_button.menu

        Windows_button=Menubutton(self.menubar,text = "Windows", underline = 0)
        Windows_button.pack(side=RIGHT)
        Windows_button.menu = Menu(Windows_button)
        Windows_button.menu.add_command(label="Analyse␣Experimental␣Data", command=self.analysedwin)
        Windows_button['menu'] = Windows_button.menu


    def makeMainframe(self):

        self.postures = ('restlessness','kicking','stamping',' rolling ','jumping','teat␣seeking',
                      'easing␣quarters',' licking / biting␣site',' tail ␣wagging','hyperventilating','trembling')
                      #exploratory behaviour, lamb/lamb interaction,suckling,lamb/mother interaction

        self.timevar=IntVar()
        self.maxtime=180 #maximum time on slider scale
        self.awake=StringVar()
        self.vocal=StringVar()
        self.recumb=StringVar() #V1,S1 etc
        self.posture=StringVar()
        self.evoked=StringVar()
        self.evokedvar=IntVar()
        self.clocktimevar=StringVar()

        self.mainframe=Frame(self)
        self.mainframe.pack(expand=YES,fill=X)
        self.botframe=Frame(self.mainframe) #frame to put activity map in
        self.botframe.pack(side=BOTTOM,expand=YES,fill=X)

        self.mapframe= Frame(self.botframe)
        self.mapframe.pack(side=LEFT)


        self.timeframe= Frame(self.mainframe,relief=RIDGE,bd=2)
        self.timeleft = Frame(self.timeframe)
        self.timeright= Frame(self.timeframe, relief=RIDGE,bd=2)
        self.timeframe.pack(expand=YES,fill=X,anchor=NW)
        self.timeleft.pack(expand=YES,fill=X,anchor=NW,side=LEFT)
        self.timeright.pack(side=RIGHT)

        self.timeslider = Scale(self.timeleft, from_=1, to=self.maxtime,   orient=HORIZONTAL, length="3i",
```

```
                        tickinterval =10,     label="time(minutes)",
                        font=("Helvetica" , 10),   command=self.entryset,width=7)
self . timeslider .pack(expand=YES,fill=X)

Label(self.timeright, text='time_(minutes)',font=("Helvetica" , 10)). pack()
self .time_entry = Entry(self . timeright ,width=7,textvariable=self.timevar)
self .time_entry.pack(expand=NO,anchor='n')
Label(self.timeright, text='clock_time', font=("Helvetica" , 10)). pack()
Label(self. timeright, textvariable=self.clocktimevar, font=("Helvetica" , 10)). pack(expand=NO) #clcck time thingy

self .time_entry.bind("<Key>",self. slideset)



self . sliderframe =Frame(self.mainframe,relief=RIDGE,bd=2)
self . sliderframe.pack(expand=YES,fill=X)

#vocalisation (no. of events)
vocalframe =Frame(self.sliderframe, relief =RIDGE,bd=2)
Menubutton(vocalframe,text = "Vocalisation", underline = 0,
       font=("Helvetica" , 10)). pack(expand=NO,side=TOP)
self. vocalslider = Scale(vocalframe, from_=0, to=30, orient=VERTICAL, length="2i",
                        tickinterval =10,width=7 ,font=("Helvetica" , 10))
self . vocalslider .pack(expand=NO,side=BOTTOM)
vocalframe.pack(expand=YES,side=LEFT,fill=X)

for key in [ '<v>','<w>','<r>', '<k>', '<s>', '<o>', '<j>', '<l>' ]:
     self.master.bind("%s"%key,self.slideincr,"+")

#restlessness slider — up and down event
restlessframe=Frame(self.sliderframe, relief =RIDGE,bd=2)
Menubutton(restlessframe,text = "Restlessness", underline = 0,font=("Helvetica" , 10)). pack(expand=NO,side=TOP)
self. restlessslider =Scale(restlessframe, from_=0, to=10, orient=VERTICAL, length="2i",
                        tickinterval =2,width=7,font=("Helvetica" , 10))
self. restlessslider .pack(expand=NO,side=BOTTOM)
restlessframe.pack(expand=YES,side=LEFT,fill=X)

#kicking slide
kickframe=Frame(self.sliderframe, relief =RIDGE,bd=2)
Menubutton(kickframe,text = "Kicking", underline = 0, font=("Helvetica" , 10)). pack(expand=NO,side=TOP)
self. kickslider =Scale(kickframe, from_=0, to=8, orient=VERTICAL, length="2i",
                        tickinterval =2,width=7,font=("Helvetica" , 10))
self. kickslider .pack(expand=YES,side=BOTTOM)
kickframe.pack(expand=YES,side=LEFT,fill=X)

#stamping slider
stampframe=Frame(self.sliderframe,relief=RIDGE,bd=2)
Menubutton(stampframe,text = "Stamping", underline = 0,font=("Helvetica", 10)).pack(expand=NO,side=TOP)
self .stampslider=Scale(stampframe, from_=0, to=8, orient=VERTICAL, length="2i",
                        tickinterval =2,width=7,font=("Helvetica" , 10))
self .stampslider.pack(expand=YES,side=BOTTOM)
stampframe.pack(expand=YES,side=LEFT,fill=X)

# rolling slider
rollframe=Frame(self.sliderframe, relief =RIDGE,bd=2)
Menubutton(rollframe,text = "Rolling", underline = 1, font=("Helvetica" , 10)). pack(expand=NO,side=TOP)
```

```
self . rollslider =Scale(rollframe, from_=0, to=8,  orient=VERTICAL, length="2i",
                     tickinterval =2,width=7,font=("Helvetica" , 10))
self . rollslider .pack(expand=YES,side=BOTTOM)
rollframe.pack(expand=YES,side=LEFT,fill=X)

#jump slider
jumpframe=Frame(self.sliderframe,relief=RIDGE,bd=2)
Menubutton(jumpframe,text = "Jumping", underline = 0,font=("Helvetica" , 10)).pack(expand=NO,side=TOP)
self.jumpslider=Scale(jumpframe, from_=0, to=8, orient=VERTICAL, length="2i",
                     tickinterval =2,width=7,font=("Helvetica" , 10))
self.jumpslider.pack(expand=YES,side=BOTTOM)
jumpframe.pack(expand=YES,side=LEFT,fill=X)

# lickbite  slider
lickbiteframe=Frame(self.sliderframe, relief =RIDGE,bd=2)
Menubutton(lickbiteframe,text = "Lick/Bite" , underline = 0, font=("Helvetica" , 10)). pack(expand=NO,side=TOP)
self . lickbiteslider =Scale(lickbiteframe , from_=0, to=8,  orient=VERTICAL, length="2i",
                     tickinterval =2,width=7,font=("Helvetica" , 10))
self . lickbiteslider .pack(expand=YES,side=BOTTOM)
lickbiteframe .pack(expand=YES,side=LEFT,fill=X)

#tailwag  slider
tailwagframe=Frame(self.sliderframe, relief =RIDGE,bd=2)
Menubutton(tailwagframe,text = "Tail_Wagging", underline = 5,font=("Helvetica" , 10)). pack(expand=NO,side=TOP)
self . tailwagslider =Scale(tailwagframe, from_=0, to=30,  orient=VERTICAL, length="2i",
                     tickinterval =5,width=7,font=("Helvetica" , 10))
self . tailwagslider .pack(expand=YES,side=BOTTOM)
tailwagframe.pack(expand=YES,side=LEFT,fill=X)



#Subjective type check boxes
self .teatseekvar = IntVar ();    self .easquartvar= IntVar()
self .hyperventvar= IntVar ();    self .tremblevar= IntVar()
self .stretchvar=IntVar()
checkframe =Frame(self.sliderframe,relief=RIDGE,bd=2)
Checkbutton(checkframe, text ="teat_seeking",
       variable=self .teatseekvar,font=("Helvetica" , 10)). pack(side=TOP)
Checkbutton(checkframe, text ="easing_quarters",
       variable=self .easquartvar,font=("Helvetica" , 10)). pack(side=TOP)
Checkbutton(checkframe, text ="hyperventilating",
       variable=self . hyperventvar,font=("Helvetica" , 10)). pack(side=TOP)
Checkbutton(checkframe, text ="trembling",
       variable=self .tremblevar,font=("Helvetica" , 10)). pack(side=TOP)
Checkbutton(checkframe, text ="stretching",
       variable=self . stretchvar , font=("Helvetica" , 10)). pack(side=TOP)
self . other_activitiesentry  = Entry(checkframe)
self . other_activitiesentry .pack(expand=YES,side=TOP)
checkframe.pack(expand=YES,side=LEFT,fill=BOTH)


#COMMENTS
Label(checkframe, text ="Comments",font=("Helvetica", 12)).pack(side=TOP)
self .commbox = Text(checkframe, height=3,width=20)
self .commbox.pack(expand=NO,side=TOP)
```

```
#get widget name so can prevent binding of sliders to keys
self.commid = self.commbox.winfo_name()
self.otheractid = self.other_activitiesentry.winfo_name()

# RECUMBENCIES

self.recumbframe = Frame(self.botframe,relief=RIDGE,bd=2)
Label(self.recumbframe,text='Recumbency',width=15,font=("Helvetica", 14)).pack(side=TOP,expand=NO)
self.recumb_lyingframe=Frame(self.recumbframe)
Label(self.recumb_lyingframe,text='Lying').pack(expand=NO,side=TOP)
Radiobutton(self.recumb_lyingframe,text='Normal−␣Head␣Up−␣V1',variable=self.recumb,value='V1',
        anchor=W,font=("Helvetica", 10)).pack( fill =X)
Radiobutton(self.recumb_lyingframe,text='Normal−␣Head␣down−␣V2',
        variable=self.recumb,value='V2',anchor=W,font=("Helvetica", 10)).pack( fill =X)
Radiobutton(self.recumb_lyingframe,text='Abnormal−␣partial␣leg␣extension−␣V3',
        variable=self.recumb,value='V3',anchor=W,font=("Helvetica", 10)).pack( fill =X)
Radiobutton(self.recumb_lyingframe,text='Abnormal−␣full␣leg␣extension−␣V4',
        variable=self.recumb,value='V4',anchor=W,font=("Helvetica", 10)).pack( fill =X)

Radiobutton(self.recumb_lyingframe,text='Lateral␣lying␣head␣up−␣L1',
        variable=self.recumb,value='L1',anchor=W,font=("Helvetica", 10)).pack( fill =X)
Radiobutton(self.recumb_lyingframe,text='Lateral␣lying␣head␣down␣−␣L2',
        variable=self.recumb,value='L2',anchor=W,font=("Helvetica", 10)).pack( fill =X)
self.recumb_lyingframe.pack(side=LEFT,anchor=N)

self.recumb_standingframe=Frame(self.recumbframe)
Label(self.recumb_standingframe,text='Standing␣or␣Walking').pack(expand=NO,side=TOP)
Radiobutton(self.recumb_standingframe,text='Normal−␣S1',
        variable=self.recumb,value='S1',anchor=W,font=("Helvetica", 10)).pack( fill =X)
Radiobutton(self.recumb_standingframe,text='Slightly␣Abnormal−␣S2',
        variable=self.recumb,value='S2',anchor=W,font=("Helvetica", 10)).pack( fill =X)
Radiobutton(self.recumb_standingframe,text='Grossly␣Abnormal−␣S3',
        variable=self.recumb,value='S3',anchor=W,font=("Helvetica", 10)).pack( fill =X)
Radiobutton(self.recumb_standingframe,text='Immobile␣(<10s)␣−␣SS1',
        variable=self.recumb,value='SS1',anchor=W,font=("Helvetica", 10)).pack(fill=X)
Radiobutton(self.recumb_standingframe,text='Immobile␣(>10s)␣−␣SS2',
        variable=self.recumb,value='SS2',anchor=W,font=("Helvetica", 10)).pack(fill=X)
self.recumb.set('V1')
self.recumb_standingframe.pack(side=RIGHT,anchor=N)
self.recumbframe.pack(side=LEFT,expand=YES,fill=BOTH)

#Objective type sliders
#consciousness slider from fully awake to asleep
##0 − eyes open full awake, 2− eyes partly closed, 3 eyes closed but awake
## 4−eyes closed asleep
awakeframe=Frame(self.botframe,relief=RIDGE,bd=2)
Label(awakeframe,text="Lack␣of\nConsciousness",font=("Helvetica", 10)).pack(expand=NO,side=TOP)
self.awakeslider=Scale(awakeframe, from_=0, to=4, orient=VERTICAL, length="2i",
                    tickinterval =1,width=7,font=("Helvetica" , 10))
self.awakeslider.pack(expand=NO,side=TOP)
awakeframe.pack(expand=YES,side=LEFT,fill=Y)

#evoked response
evokedframe=Frame(self.botframe,relief=RIDGE,bd=2)
Label(evokedframe,text="Evoked\nResponse",font=("Helvetica", 10)).pack(expand=NO,side=TOP)
```

```
        self .evokedslider=Scale(evokedframe, orient=VERTICAL, from_=0, to=100, length="2i",
                          tickinterval =25,width=7,font=("Helvetica" , 10))
        self .evokedslider.pack(expand=NO,side=BOTTOM)
        evokedframe.pack(expand=YES,side=LEFT,fill=Y)



        #MAKE MAP
        self . mapwidth =100
        self . mapheight =100
        self . realwidth =4000
        self . realheight =6000

        self . lambcoordlabel = StringVar()
        self . mothercoordlabel = StringVar()
        self . makeYardMap()


    def makeYardMap(self):
        """make␣map␣window␣and␣label␣coordinates"""
        self . mapcanv = yardmap.mapbase(self.mapframe,self.mapwidth,self.mapheight,
                self . realwidth, self . realheight )
        self . mapcanv.pack()
        yardmap.mapbase.makeNewDot(self.mapcanv, 10,10)
        self . coordframe= Frame(self.mapframe)
        self . coordframe.pack()
        yardmap.coordwidget(self.coordframe,self.lambcoordlabel,self.mothercoordlabel)
        # update coord label
        yardmap.mapbase.getCoords(self.mapcanv,self.lambcoordlabel,self.mothercoordlabel)
        #bind mouse movement to update of x and y co−ords
        self .mapcanv.bind("<ButtonRelease−1>", lambda s=self, sm=self.mapcanv,
            lam=self.lambcoordlabel, mother=self.mothercoordlabel : yardmap.mapbase.getCoords(sm,lam,mother),"+")

    def makeButtonBar(self):
        widget.ButtonBar(self ,[( ' Print ' , self . printit ),( 'Write␣Data',self . savedata)],
                [( 'Quit' , self . quit )]). pack(side=BOTTOM, fill=X)

    def printit ( self ):
        """whats␣this␣for␣then?"""
        self . datadict = self . getdata()
        print self . datadict

    def getdata( self ):
        """get␣all␣variables␣from␣single␣reading␣and␣return␣dictionary"""
            #pop up something so it's obvious button has been activated

        self . bell ()

        print self . alarmonvar.get()

        if self . alarmonvar.get(): # place call to ring bell at next timeincr
            try :
                self . after_cancel( self . alarmid) # remove call if entered before beep
            except: pass
            self . alarmid = self . after ( self . timeincr * 60000, self . bell )
```

```
self.time = self.timevar.get()

 # THE SLIDERS
self.vocalise= self.vocalslider.get()
self.restless = self.restlessslider.get()
self.kick = self.kickslider.get()
self.stamp= self.stampslider.get()
self.roll  = self.rollslider.get()
self.jump = self.jumpslider.get()
self.lickbite = self.lickbiteslider.get()
self.tailwag = self.tailwagslider.get()

#CHECK BOXES — will return zero or 1
self.teatseek = self.teatseekvar.get()
self.easquart= self.easquartvar.get()
self.hypervent = self.hyperventvar.get()
self.tremble = self.tremblevar.get()

self.stretch=  self.stretchvar.get()
self.activities = self.other_activitiesentry.get()
if self.stretch:     ##put stretch into activities field
    self.activities = 'stretch,␣%s'%self.activities
self.conscious = self.awakeslider.get()
self.recumbency = self.recumb.get()
self.comment=string.join(string.split(self.commbox.get(1.0,END)))

#put in data dictionary
datadict = {}
datadict['time']= self.time
datadict['vocalise']= self.vocalise
datadict['restless']=self.restless
datadict['kick']=self.kick
datadict['stamp']=self.stamp
datadict['roll']=self.roll
datadict['jump']=self.jump
datadict['lickbite']=self.lickbite
datadict['tailwag']=self.tailwag
datadict['conscious']= self.conscious
datadict['teatseek']= self.teatseek
datadict['easquart']= self.easquart
datadict['hypervent']= self.hypervent
datadict['tremble']= self.tremble
datadict['activities']= self.activities

datadict['recumbency']= self.recumbency
datadict['lambmap']= self.lambcoordlabel.get()
datadict['mothermap']= self.mothercoordlabel.get()
datadict['comment']= self.comment
self.commbox.delete(1.0,END)

for d in datadict.keys(): # put in NULL where fields empty
    if not datadict[d]:
        del(datadict[d]) # = 'NULL'
```

```
        if self.evokedslider.get():  #if evoked response was recorded
            datadict['evokedresponse']= self.evokedslider.get()
            self.evokedslider.set(0)


        print datadict
        return datadict

    def savedata(self):
#       """Append data to file """
        #open file , append line to file
        # try:
            self.datadict = self.getdata()     #get all the behavioural variables from screen
            datameth.data_append(self.datafile,self.datadict)
            self.updatedisplay()
        # except(AttributeError):
        #   self.nofile()

    def nofile(self):
        Dialog(self, title ='No␣File',
                        text ="Open␣a␣file␣first!",
                        bitmap = 'error',
                        default=0, strings = ("OK",))

    def openfile(self):
        """Append␣data␣to␣exisiting␣file"""
        d = LoadFileDialog(self)
        self.datafile =d.go(dir_or_file =os.getcwd() ,key="file")

        self.time = datameth.getlasttime(self.datafile ) #make new self.time
        self.updatedisplay()
        #load config details from file header
        self.configdict= datameth.get_config(self.datafile )
        self.updateconfig()
        self.master.title ("Behavioural␣Recorder␣−␣%s␣%s"%(self.configdict['sheepid'],
                self.configdict['date']))

    def printhtdata(self):
        try:
            htname =os.path.splitext(self.datafile )[0]+'.html'
            print htname
            htdata = htmlprint.DataPrint(self.datafile )
            htout=open(htname,'w')
            htout.write('%s'%htdata)
        except(AttributeError): self.nofile()

    def updateconfig(self):
        """Update␣all␣config␣vars␣when␣new␣file␣loaded"""
        self.clockstart=self.configdict['clock']
        #place critters in right map coords
        lastdic=datameth.getlastline(self.datafile )

        for n in ['mothermap', 'lambmap']:
          # reo=re.search ('\'([^,]+)..?(\ d+)', lastdic[n] )
            x,y =string.split (lastdic[n], ',' )
```

```
            print "x—%s"%x
            print "y—%s"%y
            x = ( float (x)/ self . realwidth ) * self . mapwidth
            y = ( float (y)/ self . realheight ) * self . mapheight
            if n == 'mothermap':
                yardmap.moveDot(self.mapcanv, x,y,"mother")
            elif n == 'lambmap':
                yardmap.moveDot(self.mapcanv, x,y,"lamb")
        # update coord labels
        yardmap.mapbase.getCoords(self.mapcanv,self.lambcoordlabel,self.mothercoordlabel)

    def updatedisplay(self ):
        """update␣display␣sliders␣etc.␣rezero"""
        self . timevar. set( self . time+self . timeincr)
        self . timeslider . set( self . time+self . timeincr)
        self . vocalslider . set(0)
        self . restlessslider . set(0)
        self . kickslider . set(0)
        self . stampslider. set(0)
        self . rollslider . set(0)
        self . jumpslider. set(0)
        self . lickbiteslider . set(0)
        self . tailwagslider . set(0)

        self . teatseekvar. set(0)
        self . easquartvar. set(0)
        self . hyperventvar. set(0)
        self . tremblevar. set(0)
        self . stretchvar . set(0)
        self . other_activitiesentry . delete(0,END)




##############
# MAP MAKING STUFF
#############





##########
#TIME SLIDER
##########

    def make_clocktime(self, clockstart , plusminutes):
        """Add␣minutes␣on␣to␣the␣clock␣time␣and␣return␣in␣hh:mm"""
        starthr , startmin=string . split ( clockstart , ' :' )
        ##convert whole thing tominutes
        startminutes=( int ( starthr )*60) + int (startmin ) + int (plusminutes)
        hr , min = divmod(startminutes,60)
        day,hr = divmod(hr,24) # to get 24hr clock turnover
        clocktime = string . join ([' hr ', string . zfill (min ,2)], ' :' )
        return clocktime
```

```
    def entryset(self,val):
        self.timevar.set(val)

        #clock display stuff
        self.clocktime=self.make_clocktime(self.clockstart,val)
        self.clocktimevar.set(self.clocktime)

####################
# BINDINGS
######################

    def slideset(self,event):
        t=self.time_entry.get()
        self.timeslider.set(t)


    def slideincr(self,event):
        """Bindings for activity sliders"""
        print event.keysym
        wid = self.focus_get().winfo_name()
        #if the focus is in comments or other activities dont increment slider
        if (wid == self.otheractid) or (wid == self.commid):
            return
        if event.keysym == 'w': #tail wag
            self.tailwagslider.set(self.tailwagslider.get() +1)
        elif event.keysym == 'v':    #vocalise
            self.vocalslider.set(self.vocalslider.get()+1)
        elif event.keysym == 'r':    # restless
            self.restlessslider.set(self.restlessslider.get()+1)
        elif event.keysym == 'k':    # kicking
            self.kickslider.set(self.kickslider.get()+1)
        elif event.keysym == 's':    # stamping
            self.stampslider.set(self.stampslider.get()+1)
        elif event.keysym == 'o':    # rolling
            self.rollslider.set(self.rollslider.get()+1)
        elif event.keysym == 'j':    # jumping
            self.jumpslider.set(self.jumpslider.get()+1)
        elif event.keysym == 'l':    # licking biting
            self.lickbiteslider.set(self.lickbiteslider.get()+1)


        self.bell()


#####################
#
#CONFIGURATION UTITLITIES
#
######################
    def details(self):
        """details␣configuration␣window,␣will␣add␣sheepID␣and␣date␣or␣increment␣to␣get
        unique experiment ID for joining database"""
        castration=['rings','cold␣knife','burdizzo']
        tail_docking = ['rings','cold␣knife','hot␣knife','burdizzo','burdizzo/knife']
```

```
analgesic= ['xylazine', 'lignocaine', 'flunixin␣meglumine']
self.details_win = Toplevel()
self.details_win.title ("Detail␣Entry")
mainframe=Frame(self.details_win)
mainframe.pack()

sheepdateframe=Frame(mainframe)

Label(sheepdateframe, text='Sheep␣ID').pack(side=LEFT)
sheepid_entry = Entry(sheepdateframe, textvariable=self.sheepid,width=7)
sheepid_entry.pack(side=LEFT)
Label(sheepdateframe, text='Date',width=7).pack(side=LEFT)
date_entry  =Entry(sheepdateframe ,textvariable=self.dateentry,width=9)
date_entry.pack(side=LEFT)
Label(sheepdateframe, text='Clock␣Time').pack(side=LEFT)
clockstartentry= Entry(sheepdateframe,textvariable=self.clockstartvar,width=9)
clockstartentry.pack(side=LEFT)
Label(sheepdateframe , text='Sex',width=7).pack(side=LEFT)
Radiobutton(sheepdateframe ,text='male',value="male",
        variable=self.sex,anchor=NW).pack(side=LEFT)
Radiobutton(sheepdateframe ,text='female',value="female",
        variable=self.sex,anchor=NW).pack(side=LEFT)
sheepdateframe.pack(fill=X)

procedframe = Frame(mainframe)
procedframe.pack(fill=X)
commentframe=Frame(mainframe)
commentframe.pack(fill=X)
Label(procedframe, text='Treatments').pack()
eartagframe =Frame(procedframe,relief=RIDGE,bd=2)
eartagframe.pack(side=LEFT,expand=YES,fill=BOTH)
tailframe=Frame(procedframe,relief=RIDGE,bd=2)
tailframe.pack(side=LEFT,expand=YES,fill=BOTH)
castframe=Frame(procedframe,relief=RIDGE,bd=2)
castframe.pack(side=LEFT,expand=YES,fill=BOTH)
mulesframe=Frame(procedframe,relief=RIDGE,bd=2)
mulesframe.pack(side=LEFT,expand=YES,fill=BOTH)
analgframe=Frame(procedframe,relief=RIDGE,bd=2)
analgframe.pack(side=LEFT,expand=YES,fill=BOTH)

Label(eartagframe, text='Ear␣Tagging').pack()
Label( tailframe , text='Tail␣Docking').pack()
Label(castframe , text='Castration' ).pack()
Label(mulesframe , text='Mulesing').pack()
Label(analgframe , text='Analgesic').pack()

Checkbutton(eartagframe,variable=self.eartagvar,text="ear␣tagged",onvalue="yes").pack()

for t in tail_docking:
   Radiobutton(tailframe, text=t, variable=self.taildockvar,value=t,anchor=NW).pack(fill=X)
Radiobutton(tailframe, text='None',variable=self.taildockvar,value=None,anchor=NW).pack(fill=X)

for c in castration:
   Radiobutton(castframe, text=c,variable=self.castratevar,value=c,anchor=NW).pack(fill=X)
Radiobutton(castframe, text='None',variable=self.castratevar,value=None,anchor=NW).pack(fill=X)
```

```
        Checkbutton(mulesframe,text="Mulesed",variable=self.mulesingvar,onvalue="yes").pack()

        for a in analgesic:
            Radiobutton(analgframe, text=a,variable=self.analgesicvar,value=a,anchor=NW).pack(fill=X)
        Radiobutton(analgframe, text='None',
                        variable=self.analgesicvar,value=None,anchor=NW).pack(fill=X)
        Entry(analgframe,textvariable=self.analgesicvar).pack( fill =X)

        Label(analgframe,text="Dose").pack( fill =X)
        Entry(analgframe,textvariable=self.analgdosevar).pack( fill =X)

        Label(analgframe,text="Administration").pack( fill =X)
        for adm in ['i.m.', 'i.v.', 'i.p', 'oral' ]:
                Radiobutton(analgframe, text=adm,variable=self.analgadmvar,
                        value=adm,anchor=NW).pack(fill=X)

        Label(commentframe, text="Observer").pack()
        observerentry= Entry(commentframe, textvariable=self.observervar)
        observerentry.pack()

        Label(commentframe, text="Comments").pack(fill=X)
        self.detailscommentstext=Text(commentframe,width=20,height=3)
        self.detailscommentstext.pack( fill =X)


    def newfile( self ):
        self.details ()
        widget.ButtonBar(self.details_win ,[( 'Create␣File', self.config_get )],
              [( 'Close', self.details_win.destroy )]).pack(side=BOTTOM,fill=X)



    def table_alter ( self ):
        self.details ()
        self.detailscommentstext.insert(1.0, self.detailscomments)
        widget.ButtonBar(self.details_win ,[( 'Change␣Details',self.config_get )],
             [( 'Close', self.details_win.destroy )]). pack(side=BOTTOM,fill=X)

    def config_get( self ):
        """get␣config␣details"""
        self.sheepid =self.sheepid.get()
        self.date = self.dateentry.get()
        self.gender=self.sex.get() #lambs gender
        self.eartag = self.eartagvar.get()
        self.taildock=self.taildockvar.get()
        self.castrate=self.castratevar.get()
        self.mulesing=self.mulesingvar.get()
        self.analgesic = self.analgesicvar.get()
        self.analgesicdose=self.analgdosevar.get()
        self.observer = self.observervar.get()
        self.clockstart=self.clockstartvar.get()
        self.analgesicroute = self.analgadmvar.get()
        self.detailscomments= string.join( string. split ( self.detailscommentstext.get(1.0,END)))

        self.configdict ={}
```

```
        self . configdict [ 'sheepid' ] = self .sheepid
        self . configdict [ 'date' ] = self .date
        self . configdict [ 'sex' ]= self .gender
        self . configdict [ ' taildock ' ]= self . taildock
        self . configdict [ ' castration ' ]=self . castrate
        self . configdict [ 'mulesing']=self.mulesing
        self . configdict [ 'analgesic']=self . analgesic
        self . configdict [ 'analgesicdose']= self . analgesicdose
        self . configdict [ 'analgesicroute']= self . analgesicroute
        self . configdict [ 'eartag']=self . eartag
        self . configdict [ 'observer']=self . observer
        self . configdict [ 'comments']= self.detailscomments
        self . configdict [ 'clock' ] = self . clockstart
        self . configdict [ 'observer'] = self . observer

        print self . configdict
        self . expid = datameth.createexpID(self.configdict)
        self . configdict [ 'expid']=self . expid
        self . savedatadir = datameth.savedatadir(self)
        print self . savedatadir

        self . datafile =datameth.createfilename(self.expid, self . savedatadir)
        datameth.filenew(self. datafile , self . configdict ) #write config and detail data to file
        self . details_win . destroy()

        self . master. title ("Behavioural␣Recorder␣−␣%s␣%s"%(self.sheepid,self.date))

####################
# SETUP
####################
    def setup(self ):
        """Setup␣window␣for␣display␣options"""


        self . setup_win = Toplevel()
        self . setup_win. title ("Setup")
        setupframe= Frame(self.setup_win)
        setupframe.pack(expand=YES,fill=BOTH)
        Label(setupframe,text="maximum␣time␣on␣slider").grid(row=0,column=0)
        Entry(setupframe,textvariable =self . maxtimevar).grid(row=0,column=1)
        self . maxtimevar.set(self.maxtime)
        Label(setupframe,text="distance␣of␣x␣axis␣in␣map").grid(row=1,column=0)
        self . realwidthvar. set( self . realwidth)
        self . realheightvar. set( self . realheight)
        Entry(setupframe,textvariable =self . realwidthvar). grid (row=1,column=1)
        Label(setupframe,text="distance␣of␣y␣axis␣in␣map").grid(row=2,column=0)
        Entry(setupframe,textvariable =self . realheightvar). grid (row=2,column=1)
        Label(setupframe,text="clock␣time␣start"). grid (row=3,column=0)
        self . clockstartvar . set( self . clockstart )
        Entry(setupframe,textvariable =self . clockstartvar ). grid (row=3,column=1)
        self . timeincrvar . set( self . timeincr)
        Label(setupframe,text="Auto␣increment␣for␣each␣entry").grid(row=4,column=0)
        Entry(setupframe,textvariable =self . timeincrvar ). grid (row=4,column=1)
        Label(setupframe,text="Alarm").grid(row=5,column=0)
        Checkbutton(setupframe,text="On", variable=self.alarmonvar).grid(row=5,column=1)
```

```
        widget.ButtonBar(self.setup_win,[('Apply', self.setup_get)],
                [('Close', self.setup_win.destroy)]).pack( fill =X,expand=NO)

    def setup_get(self):
        """␣GEt␣setup␣details"""
        self.maxtime=self.maxtimevar.get()
        self.timeslider.config(from_=0, to=self.maxtime)#redraw slider
        self.realwidth = self.realwidthvar.get()
        self.realheight = self.realheightvar.get()
        # rescale canvas , forget packing and then redraw
        self.mapcanv.pack_forget()
        self.coordframe.pack_forget()
        self.makeYardMap()

        self.clockstart=self.clockstartvar.get()
        self.timeincr=self.timeincrvar.get()

    def analysedwin(self):
        """open␣window␣with␣analysed␣data"""
        #try:
        datawin.Analyzed(self.datafile)
        #except: self.nofile() # no file open yet

if __name__ == '__main__':
    Recorder().mainloop()
```

10.1.2 Data handling methods

10.1.2.1 datameth.py

Listing 10.2: Data and file handling methods

```
#!/usr/bin/env python
#
# some methods for data and file handling

import os, string
from FileDialog import *
import math, re

datafields = ('time','recumbency','vocalise','restless', 'lickbite',
 'tailwag','stamp', 'roll', 'jump', 'kick', 'teatseek', 'easquart',
 'hypervent', 'tremble','stretch',' activities ','evokedresponse',
 'conscious','lambmap', 'mothermap','comment')

def data_append(filename,datadict):
    """␣append␣data␣to␣file"""
    f = open(filename,'a')
    f.write('%s\n'%datadict)
    f.close()
```

```
def filenew(filename, configdict ):
    """create␣new␣data␣file␣and␣write␣experimental␣details␣at␣head"""

    f =open(filename,'w')
    for k in configdict.keys():
      f.write('%s␣:␣%s\n' % (k, configdict[k]))

    datadelimit = '#'*45
    f.write('%s\n'%(datadelimit))
    f.close()


def getlastline (filename):
    """Get␣last␣line␣and␣return␣as␣a␣dictionary␣based␣on␣datafield␣names"""
    f=open(filename)
     linelist = f.readlines()
    ## what if its blank?
    lastpos = −1
    while 1:
        lastline = linelist [lastpos]
        if len( lastline ) > 2:
            break
        lastpos=lastpos−1
    linedic=eval( lastline )

    return linedic

def getlasttime(filename):
    """get␣last␣line␣and␣return␣time␣etc,␣could␣use␣method␣abive␣to␣get␣time"""
     linedic = getlastline (filename)
    time = linedic['time']
    return int(time)

def createexpID(configdict):
    """try␣and␣create␣a␣unique␣experimental␣ID␣from␣config␣details"""
    tailmeths=['','rings','cold␣knife','hot␣knife','burdizzo','burdizzo/knife']
    castmeths =['','rings','cold␣knife','burdizzo','burdizzo/knife']

    expid = configdict['sheepid'] +'−'+configdict['date'] +'−'

    if len(configdict['taildock'])>1: #use index position for tail dock id
        expid = expid+'tailmeths.index(configdict['taildock'])'
    else: expid = expid+ '0'

    if len(configdict['castration']) > 1:
        expid = expid+'castmeths.index(configdict['castration'])'
    else: expid = expid+ '0'

    if len(configdict['mulesing']) > 1:
        expid = expid+'m'
    if len(configdict['eartag']) > 1:
          expid = expid+'e'
    if len(configdict['analgesic']) >1:
        expid = expid+ configdict['analgesic'][:3]
```

```
    return expid


def createfilename(expid,directory=' ./' ):
    """make␣a␣file␣name␣based␣on␣expID␣and␣work␣out␣its␣location
    if there is a default directory"""
     file_ext = ".bhv"
    filename = expid + file_ext
    fullname = os.path. join (directory ,filename)

    #if filename already exists return question if wanting to overwrite file
    if os.path.exists (fullname):
       print "I␣am␣overwriting␣this␣file␣OK?"

    return fullname


def savedatadir(mainwin):
    """returns␣directory␣to␣save␣data␣to"""
    #this needs to be changed to something that works
    save_d= LoadFileDialog(mainwin)
    filename=save_d.go(dir_or_file=os.getcwd(),key="file")
    datadir = os.path. split (filename)[0] #just return directory
    return datadir


############################
# TRAVEL MAP STUFF
##########################


def hypotlength(coords1, coords2):
    """return␣the␣length␣from␣right␣angle␣triangle␣calculations␣where
    length = sqrt(a^2 + b^2).  coords are tuples ie distance from on point
    to other"""

    x1,y1 = coords1
    x2,y2 = coords2
    a= x1 - x2 # a is length of x aspect of triangle
    b = y1 - y2 # b is length of y aspect of triangle
    return math.hypot(a,b)

def traveldistance(coords):
    """Calculate␣total␣travel␣distance␣from␣a␣list␣of␣map␣coords␣as␣tuples"""
     totdist =0
    reo1= re.compile(' ([\w |\.]+).*?,\ s *([\w |\.]+)' ) # this could be improved

    for c in coords:
        cpairs=reo1.search(c)
        cfloat = map(float, cpairs.groups()) # turn strings into values

        try :
              totdist = hypotlength(cfloat , lastc ) + totdist
        except: pass # for first set of coords
        lastc = cfloat
```

```
    return totdist



def map2data(coords):
    """convert␣list␣of␣map␣pairs␣into␣list␣of␣x␣and␣y's"""
    xdata =[]
    ydata =[]
    reo1= re.compile(' ([\w |\.]+).*?,\ s *([\w |\.]+)' )

    for c in coords:
        cpairs=reo1.search(c)
        cfloat = map(float, cpairs.groups()) # turn strings into values
        xdata.append(cfloat[0])
        ydata.append(cfloat[1])
    return [xdata,ydata]


#############################################
#  Output/Printing methods
#############################################

def print_file (configdict, datadict):
    """Fancy␣print␣output␣for␣printing␣hard␣copy␣of␣data"""

    # get max length of each fields for justification
    fieldlength = {} # dictionary to hold maximum field lengths
    times = sort_textby_int(datadict.keys())
    padvalue = 2 # number of spaces to pad string by

    for t in datadict.keys(): # for each time
            for d in datadict[t].keys():  # for each field in each time
                try:
                    fieldlength [d] = max([fieldlength[d],len(datadict[t][d])])
                except:
                    fieldlength [d]=len(d) # get length of title

    for d in datafields : #print header
        print string.rjust(d, fieldlength [d]+padvalue),
    print # end line
    print '#'*60

    for t in times:
      for d in datafields:
        print string.rjust(datadict['%s'%t]['%s'%d],fieldlength[d]+padvalue),
      print # end line

##############################################
#  FILE METHODS
##############################################

def get_config(filename):
    """␣Get␣config␣␣details␣from␣file␣header"""
    datasep = ' :'  # separator between name and values
    config_end = '#'*10 #  end of config details
```

```
    configdict={}

    f = open(filename,'r')
    for l in f.readlines():
        if string.count(l,config_end): # if we find end of config
            print "here"
            break
        try:
            name,val = string.split(l,datasep,1)
            val = string.strip(val)
            name = string.strip(name)
            configdict[name] = val
            if not (configdict[name] or configdict[name]== 0):
                configdict[name] = 'NULL'
        except: # not a valid field
            pass
        l = f.readline()
    return configdict


def get_data(filename):
    """get␣data␣from␣file,␣data␣separated␣by␣tabs␣and␣return␣as␣a␣dictionary
    within a dictionary with times as key values eg. datadict[15]['recumbency']"""
    heading_sep= '#'*10 # separator between headings and data
    config_end = '#'*10 # end of config details
    datadict={}
    f = open(filename,'r')
    l = f.readline()
    while 1:
        if string.count(l,heading_sep):
            for df in f.readlines():
                linedic = eval(df)
            # print data
                datadict[linedic['time']]= linedic # dictionary with time as key
            break
        l = f.readline()
    return datadict


def sortbylist(keylist, val_list):
    """Sort␣val_list␣based␣on␣values␣in␣keylist"""
    pairs = map(None, keylist, val_list)
    pairs.sort()
    result=pairs[:]
    for i in xrange(len(result)):
        result[i] = result[i][1]
    return result


def time_vals(datadict,var):
    """return␣list␣with␣two␣lists␣of␣xdata(time)␣and␣ydata(whatever)"""
    xdata=[]
    ydata=[]

    for time in datadict.keys():
```

```
        if var in ['vocalisation', 'consciousness', 'evokedresponse']:
            try:
                ydata.append(int(datadict[time][var]))
                xdata.append(int(time)) # this will always be a number
            except(ValueError):
                pass # Not recorded or so forth in
        else:
            xdata.append(int(time)) # this will always be a number
            ydata.append(datadict[time][var])

    ydata = sortbylist(xdata,ydata) # sort y data by time
    xdata.sort() # sort the time data
    return (xdata,ydata)

def sort_textby_int(numberlist):
    """sort␣list␣by␣converting␣to␣integer␣then␣return␣integer␣list"""
    xlist = []
    for t in numberlist:
        xlist.append(int(t))
    xlist.sort()
    return xlist

def xytuples(datadict,var):
    """return␣list␣of␣tuples␣which␣represent␣x,y␣coordinates␣for␣use␣in␣plot
    module from John Grayson book"""
    tuplelist =[]
    texttimes = datadict.keys()
    xlist = []
    for t in texttimes:
        xlist.append(int(t))
    xlist.sort()

    for x in xlist:
        if var in ['vocalise', 'conscious', 'evokedresponse']:
            try:
                y = int(datadict[x][var])
                tuplelist.append((x,y))
            except(ValueError,KeyError):
                pass # Not recorded or so forth in
        else:
            y = datadict[x][var]
            tuplelist.append((x,y))

    return tuplelist

####################################################
#           OLD DEFUNCT METHODS
#         still needed for conversion of old data
#####################################################
def oldget_headings(filename):
    """Get␣headings␣for␣columns␣from␣file,␣return␣list"""
    config_end = '-'*10 # end of config details
    f = open(filename,'r')
    l = f.readline()
    while 1:
```

```
        if string.count(l,config_end):
            headings = string.split(f.readline())
            break
    return headings

def oldget_data(filename):
    """get␣data␣from␣file,␣data␣separated␣by␣tabs␣and␣return␣as␣a␣dictionary
    within a dictionary with times as key values eg. datadict[15]['recumbency']"""
    heading_sep= '#'*10 # separator between headings and data
    config_end = '-'*10 # end of config details
    datadict={}
    f = open(filename,'r')
    l = f.readline()
    while 1:
        if string.count(l,config_end):
         headings = string.split(f.readline())
     #   print headings
        if string.count(l,heading_sep):
         for df in f.readlines():
            data = string.split(string.strip(df),'\t')
            samptime=eval(data[0])
            datadict[samptime]={} # dictionary with time as key
            for (key,val) in map(None, headings,data): # wow aren't l clever
                  try:
                      ## get rid of zero values
                      val = eval(string.strip(val))
                      if val:
                          datadict[samptime][key] = val
                  except:
                      pass    ## no value
        break
     l = f.readline()
    return datadict
```

10.1.3 Display widgets

10.1.3.1 widget.py

Listing 10.3: Some custom widgets

```
#!/usr/bin/env python
#
#
# A few commonly used widgets
#

from Tkinter import *


class ButtonBar(Frame):
   """␣A␣simple␣button␣bar␣list␣on␣left␣or␣list␣on␣right"""
```

```
    def __init__(self,master, left_button_list, right_button_list):
        Frame.__init__(self,master,bd=2,relief=SUNKEN)
        for button,action in  left_button_list :
            Button(self,  text=button,command=action).pack(side=LEFT)
        for button,action in  right_button_list :
            Button(self,  text=button,command=action).pack(side=RIGHT)

class MyScale(Scale):
    """Scale␣with␣a␣few␣presets"""
    def __init__(self,master):
        Scale.__init__(self,master)
```

10.1.3.2 yardmap.py

Listing 10.4: Travel map widgets

```
#!/usr/bin/env python
#

from Tkinter import *
import string, regsub


class mapbase(Canvas):
    def __init__(self,master,width,height,realwidth, realheight,**kw):
        self.width = width
        self.height= height
        self.mother=1 # mother in yard
        self.dotsize = 8 # dot size in pixels
        Canvas.__init__(self,master,width=self.width,height=self.height,background = 'green')

        self.pack( fill =BOTH,expand=YES)
        self.bind("<1>", self.mouseDown,"+")
        self.bind("<B1-Motion>", self.mouseMove,"+")
        self.bind("<Double-Button-1>",self.pointstogether,"+")
        self.bind("<3>", self.mapmenu,"+")
        self.realwidth = realwidth
        self.realheight = realheight

    def makeNewDot(self, xpos,ypos):
        # create a dot, and mark it as current

        lamb =self.create_oval(xpos-(self.dotsize/2),ypos-(self.dotsize/2),
              xpos+(self.dotsize/2), ypos+(self.dotsize/2), fill ="blue", tags="lamb")
        mother = self.create_oval(xpos+10-(self.dotsize/2),
              ypos+10-(self.dotsize/2),xpos+10+(self.dotsize/2),ypos+10+(self.dotsize/2),
                                      fill ="red", tags="mother")

    def mouseDown(self,event):
        if event.widget.find_withtag(CURRENT) :
            self.lastx = event.x
            self.lasty = event.y
```

```
            self.dtag("selected") # remove last selected tag
            self.addtag("selected", "withtag", CURRENT)

    def pointstogether(self,event):
        """Bring␣points␣together␣on␣map"""
        tag = event.widget.gettags(CURRENT)[0]
        tagx0,tagy0,yagx1,tagx1 = self.coords(tag) # this returns a list
        for x in self.find_all(): # for all the tags on canvas
            if tag not in self.gettags(x):
                othertag = self.gettags(x)[0]
                self.coords(othertag,tagx0,tagy0,yagx1,tagx1)   #this doesn't take a list


    def mouseMove(self,event):
         if  self.type("selected") == "oval":
            self.move("selected", event.x − self.lastx, event.y − self.lasty)
            self.lastx = event.x
            self.lasty = event.y

    def mapmenu(self, event):
        """"pop up option menu"""
        pmenu = Menu(event.widget, tearoff=0)
        pmenu.add_checkbutton(label="No␣Mother", underline=0,
                command=self.motheroff)
        pmenu.tk_popup(event.x_root, event.y_root)


    def motheroff(self):
        """Turn␣mother␣on−off"""
        if self.mother:
            self.mother=0
            self.delete("mother")
        else:
            self.mother=1 #create new dot
            mother = self.create_oval(20−(self.dotsize/2),
                20−(self.dotsize/2),20+(self.dotsize/2),20+(self.dotsize/2),
                                      fill ="red", tags="mother")


    def getCoords(self,labelvar1=None,labelvar2=None):
        """update␣all␣coord␣labels"""
        for x in self.find_all():
            tag = self.gettags(x)[0]
            coordtuple =  self.coords(tag)
            xcoord = (coordtuple[2] + coordtuple [0]) / 2
            ycoord = (coordtuple[3] + coordtuple [1]) / 2
            xcoord = self.convertcoord(xcoord,self.width,self.realwidth)
            ycoord = self.convertcoord(ycoord, self.height, self.realheight)
            if not self.mother:
                labelvar2.set('NULL')
            if tag == 'lamb' :
                labelvar1.set('%s,␣%s'%(xcoord, ycoord))
            elif tag == 'mother':
                    labelvar2.set('%s,␣%s'%(xcoord, ycoord))
```

```
    def convertcoord(self,coord,mapsize, realsize):
        """convert␣coord␣in␣pixels␣into␣value␣based␣on␣real␣width␣and␣height
        mapsize is display size eg, self.width
        realsize is self.realheight etc."""
        return (coord/mapsize) * realsize


def moveDot(self,xpos,ypos,tagname):
        """move␣dot␣from␣current␣pos␣to␣new␣pos."""

        tagx0,tagy0,tagx1,tagy1 = self.coords(tagname)
        diffx = tagx1-tagx0
        diffy = tagy1-tagy0
        self.coords(tagname,xpos-(diffx/2),
                ypos-(diffy/2),xpos+(diffx/2),ypos+(diffy/2))
        self.lastx = xpos
        self.lasty = ypos


def coordwidget(self,lamblabel,motherlabel):
    """widget␣for␣lamb␣and␣mother␣coords"""
    Label(self, text ="Lamb", font=("Helvetica", 12), fg="blue").pack()
    Label(self, textvariable=lamblabel, relief=SUNKEN,width=12).pack()
    Label(self, text ="Mother", font=("Helvetica", 12), fg="red").pack()
    Label(self, textvariable=motherlabel, relief=SUNKEN,width=12).pack()


if __name__ == '__main__':
    root = Tk()
    root.title ("Map␣Test")
    root.lambcoordlabel = StringVar()
    root.mothercoordlabel = StringVar()

    root.mapcanv = mapbase(root,200,300,400,600)
    root.mapcanv.pack()
    mapbase.makeNewDot(root.mapcanv, 10,10)
    coordframe= Frame(root)
    coordframe.pack()
    coordwidget(coordframe,root.lambcoordlabel,root.mothercoordlabel)
    #bind mouse to get coords
    root.mapcanv.bind("<Button-2>",
        lambda m=mapbase: moveDot(root.mapcanv,35,150,"mother"),"+")
    root.mapcanv.bind("<ButtonRelease-1>",
        lambda m=mapbase: mapbase.getCoords(root.mapcanv,root.lambcoordlabel,root.mothercoordlabel),"+")
    mapbase.getCoords(root.mapcanv,root.lambcoordlabel,root.mothercoordlabel)
    root.mainloop()
```

10.1.3.3 utils.py

Listing 10.5: Math functions

```
#!/usr/bin/env python


def minCoordinate(clist):
    if len( clist ) < 2: return clist [0]
    try:
        x, y = clist [0]
        for x1, y1 in clist [1:]:
            if x1 <= x or y1 <= y:
                x, y = x1, y1
    except:
        x, y = 0, 0

    return x,y

def maxCoordinate(clist):
    if len( clist ) < 2: return clist [0]
    try:
        x, y = clist [0]
        for x1, y1 in clist [1:]:
            if x1 >= x or y1 >= y:
                x, y = x1, y1
    except:
        x, y = 0, 0

    return x,y

def minBound(clist):
    x = 10000000
    y = 10000000
    for x1, y1 in clist :
        if x1 < x: x = x1
        if y1 < y: y = y1
    return x,y

def maxBound(clist):
    x = -10000000
    y = -10000000
    for x1, y1 in clist :
        if x1 > x: x = x1
        if y1 > y: y = y1
    return x,y


if __name__ == '__main__':

    tlist    = [ (5,5), (3,6), (3,3), (-2,5), (100,100),
                (100,-100), (100,101) ]

    print minCoordinate(tlist)
    print maxCoordinate(tlist)
    print maxCoordinate([(3,3), (10,-1)])
```

10.1.3.4 datawin.py

Listing 10.6: Methods for viewing and analysing data

```
#!/usr/bin/env python

#
# Windows for displaying analysed data
#

import datameth,widget
from Tkinter import *
from ScrolledText import ScrolledText
import plot

class Analyzed(Toplevel):
    def __init__(self,filename):
        Toplevel.__init__(self)
        self.configdict = datameth.get_config(filename)
        self.datadict = datameth.get_data(filename)
        self.title ("Analyzed␣Data␣%s" % filename)
        print self.datadict
        mainframe = Frame(self,bd=2,relief=RIDGE)
        mainframe.pack(expand=YES, fill=BOTH)
        Label(mainframe, text=self.configdict['sheepid']).pack()
        leftframe = Frame(mainframe,bd=2,relief=RIDGE)
        rightframe = Frame(mainframe,bd=2,relief=RIDGE)
        leftframe.pack(side=LEFT,expand=YES, fill=BOTH)
        rightframe.pack(side=RIGHT,expand=YES, fill=BOTH)

        Label(leftframe, text="Active␣pain␣behaviours").pack()
        vasplot = VAScanvas(leftframe)
        vasplot.pack()

        Label(leftframe, text="Travel␣Plot").pack()
        # extract map data from dictionary
        traveldata = datameth.time_vals(self.datadict, 'lambmap')
        print 'Total␣travel␣distance␣is␣%s' % datameth.traveldistance(traveldata[1])
        xplotdata = datameth.map2data(traveldata[1])[0]
        yplotdata = datameth.map2data(traveldata[1])[1]
        travelplot = Travelcanvas(leftframe,xdata=xplotdata,ydata=yplotdata)
        travelplot.pack()

        vochead=Frame(rightframe)
        vochead.pack(fill=X)
        vocdata=datameth.xytuples(self.datadict,'vocalise')
        Label(vochead, text="vocalization").pack(side = LEFT)
        Button(vochead, text='Show␣Data', command=lambda data=vocdata,
            text='vocalise' : Showdata(data,text)).pack(side=RIGHT)
        vocline = plot.GraphLine(vocdata,color='black', width=1, smooth=0)
        vocsym = plot.GraphSymbols(vocdata,color='blue', width=1, marker='dot')
        voc_graphobject = plot.GraphObjects([vocline,vocsym])
        vocplot = plot.GraphBase(rightframe,300,100,relief=SUNKEN,border=2)
        vocplot.pack()
```

```
        vocplot.draw(voc_graphobject,'automatic','automatic')


        conchead=Frame(rightframe)
        conchead.pack(fill=X)
        conctuples = datameth.xytuples(self.datadict, 'conscious')
        Label(conchead, text="consciousness").pack(side = LEFT)
        Button(conchead, text='Show␣Data', command=lambda data=conctuples,
                text='conciousness' :Showdata(data,text)).pack(side=RIGHT)
        #graphing stuff
        concline = plot .GraphLine(conctuples,color='black', width=1, smooth=0)
        conc_graphobject = plot.GraphObjects([concline])
        concplot = plot .GraphBase(rightframe,300,100,relief=SUNKEN,border=2)
        concplot.pack()
        concplot.draw(conc_graphobject,'automatic','automatic')

        evokedhead=Frame(rightframe)
        evokedhead.pack(fill=X)
        evokeddata = datameth.xytuples(self.datadict,'evokedresponse')
        Label(evokedhead, text="evoked␣response").pack()
        Button(evokedhead, text='Show␣Data', command=lambda data=evokeddata,
                text='evoked␣response' :Showdata(data,text)).pack(side=RIGHT)
        evokedbar=plot.GraphBars(evokeddata, color='green', size=3)
        evoked_graphobject = plot.GraphObjects([evokedbar])
        evokedplot= plot.GraphBase(rightframe,300,100,relief=SUNKEN,border=2)
        evokedplot.pack()
        evokedplot.draw(evoked_graphobject,'automatic','automatic')

        widget.ButtonBar(self ,[],[( 'Close', self .destroy )]). pack(side=BOTTOM, fill=X)


class VAScanvas(Canvas):
    """Bar␣Chart␣or␣the␣like␣of␣combined␣VAS␣scores"""
    def __init__( self ,master):
        Canvas.__init__(self,master, background = 'white')


class Travelcanvas(Canvas):
    """Travel␣map␣for␣sheep␣data,␣should␣be␣like␣Plotcanvas␣but␣be␣connected␣by
    lines and x data is in coord tuple"""
    def __init__( self ,master,xdata,ydata):
        Canvas.__init__(self,master,background = 'green')
        max_x = max(xdata)
        max_y = max(ydata)
        canvheight = int( self .config( 'height' )[4])
        canvwidth = int( self .config( 'width' )[4])
        xscale =canvwidth/max_x
        yscale = canvheight/max_y
        dotsize = 4
        mapcoords= map(None,xdata,ydata)
        lastx , lasty = mapcoords[0][0],mapcoords[0][1]

        for x,y in mapcoords:
            x=int(x)
            y=int(y)
```

```
            x=x*xscale
            y=canvheight − (y*yscale)#TO TURN UP RIGHT WAY
            self.create_line(x,y,lastx,lasty)
            self.create_oval(x−(dotsize/2),y−(dotsize/2),x+(dotsize/2),y+(dotsize/2),
                                        fill ="blue")
            lastx = x
            lasty = y

class Plotcanvas(Canvas):
    """x−y␣type␣plot␣for␣data"""
    def __init__(self,master,xdata,ydata):
        Canvas.__init__(self,master,background = 'white',height=75,width=200)
        max_x = max(xdata)
        max_y = max(ydata)
        canvheight = int(self.config('height')[4])
        canvwidth = int(self.config('width')[4])
        xscale =canvwidth/max_x
        yscale = canvheight/max_y
        dotsize = 4
        mapcoords= map(None,xdata,ydata)

        for x,y in mapcoords:
            x=int(x)
            y=int(y)
            x=x*xscale
            y=canvheight − (y*yscale)#TO TURN UP RIGHT WAY
            self.create_oval(x−(dotsize/2),y−(dotsize/2),x+(dotsize/2),y+(dotsize/2),
                                        fill ="blue")


class Showdata(Toplevel):
    """Window␣that␣opens␣to␣display␣raw␣data"""
    def __init__(self,data, title ='Data'):
        Toplevel.__init__(self)
        self. title ( title )
        mainframe = Frame(self)
        mainframe.pack()

        datatext = ScrolledText(mainframe)
        datatext.pack()
        datatext.delete(1.0,END)
        for x,y in data:
            datatext.insert(END,'%s\t%s\n'%(x,y))
        widget.ButtonBar(self,[( 'Print' , '' ),( 'Save','' )],
                [( 'Close', self.destroy )]). pack(side=BOTTOM, fill=X)
```

References

Abbott, F. and Bonder, M. (1997) Options for management of acute pain in the rat. *Veterinary Record* **140(21)**:553–557.

Anonymous (1994) Sheep welfare: FAWC's recommendations on castration, docking and sheep scab. *Veterinary Record* **134(21)**:534.

Anonymous (2001) *Wool Information Sheet*. The Vegan Society.

Baggot, J. D. (1992) Clinical pharmacokinetics in veterinary medicine. *Clinical Pharmacokinetics* **22(4)**:254–273.

Baggot, J. D. *Pharmacokinetics: Disposition and fate of drugs in the body*, In *Veterinary Pharmacology and Therapeutics* Adams, H. R., editor, chapter 4. Iowa State University Press, Ames, Iowa, Seventh edition.

Beecher, H. (1957) The measurement of pain. *Pharmacological Review* **9**:59–209.

Blackshaw, J. (1986) Objective measures of welfare in farming environments. *Australian Veterinary Journal* **63(11)**:361–364.

Bloor, B. and Schmeling, W. (1993) Cardiovascular effects of alpha 2-adrenoceptors. *Anaesthetic Pharmacology Review* **1(3)**:247–262.

Bromm, B. and Lorenz, J. (1998) Neurophysiological evaluation of pain. *Electroencephalography and Clinical Neurophysiology* **107**:227–253.

Castro, M. and Eisenach, J. (1989) Pharmacokinetics and dynamics of intravenous, intrathecal, and epidural clonidine in sheep. *Anesthesiology* **71(3)**:418–425.

Celly, C., McDonell, W. and Black, W. (1999) Cardiopulmonary effects of the alpha 2-adreoceptor agonists medetomidine and ST-91 in anesthetized sheep. *Journal of Pharmacology and Experimental Therapeutics* **289(2)**:712–720.

Celly, C., McDonell, W., Young, S. and Black, W. (1997) The comparative hypoxaemic effect of four alpha 2-adrenoceptor agonists (xylazine, romifidine, detomidine and medetomidine) in sheep. *Journal of Veterinary Pharmacology and Therapeutics* **20(6)**:464–471.

Chapman, R., Fell, L. and Shutt, D. (1994) A comparison of stress in surgically and non-surgically mulesed sheep. *Australian Veterinary Journal* **71**:243–247.

Choiniere, M. *Pain of burns*, In *Textbook of Pain* Wall, P. D. and Melzack, R., editors, chapter 28. Churchill Livingstone, Edinburgh, Third edition.

Corssen, G., Domino, E. and Sweet, R. (1964) Neuroleptanalgesia and anaesthesia. *Anesthesia and Analgesia* **43**:748–762.

de Segura, I. G., Tendillo, F., Mascias, A., Santos, M., Castillo-Olivares, J. and Steffey, E. (1997) Actions of xylazine in young swine. *American Journal of Veterinary Research* **58(1)**:99–102.

Dedrick, R. L. (1973) Animal scale-up. *Journal of Pharmacokinetics and Biopharmaceutics* **1(5)**:453–461.

Eisenach, J. (1988) Intravenous clonidine produces hypoxaemia by a peripheral alpha 2-adrenergic mechanism. *Journal of Pharmacology and Experimental Therapeutics* **244**:247–252.

Eisenach, J., Dewan, D., Rose, J. and Angelo, J. (1987) Epidural clonidine produces antinociception, but not hypotension, in sheep. *Anesthesiology* **66(4)**:496–501.

Eisenach, J., Shafer, S., Bucklin, B., Jackson, C. and Kallio, A. (1994) Pharmacokinetics and pharmacodynamics of intraspinal dexmedetomidine in sheep. *Anesthesiology* **80(6)**:1349–1359.

Ewer, T. (1942) Lamb castration and docking. *New Zealand Journal of Agriculture* **65**:337–339.

Fell, L. and Shutt, D. (1989) Behavioural and hormonal responses to acute surgical stress in sheep. *Applied Animal Behaviour Science* **22**:283–294.

Fell, L., Shutt, D. and Bentley, C. (1985) Development of a salivary cortisol method for detecting changes in plasma. *Australian Veterinary Journal* **62(12)**:403–406.

Filmer, J. (1938) Lamb tailing: a comparison of results obtained with the searing iron and knife. *Australian Veterinary Journal* **14**:189–191.

Flecknell, P. (1987) *Laboratory Animal Anaesthesia.* Academic Press, London.

French, N., Wall, R. and Morgan, K. (1994) Lamb tail docking: a controlled field study of the effects of tail amputation on health and productivity. *Veterinary Record* **134(18)**:463–467.

Graham, M., Kent, J. and Molony, V. (1997) Effects of four analgesic treatments on the behavioural and cortisol responses of 3-week-old lambs to tail docking. *Veterinary Journal* **153(1)**:87–97.

Grant, C., Upton, R. and Kuchel, T. (1996) Efficacy of intra-muscular analgesics for acute pain in sheep. *Australian Veterinary Journal* **73**:129–132.

Hall, L. and Clarke, K. (1983) *Veterinary Anaesthesia.* Bailliere Tindall, London, eigth edition.

Haskins, S., Patz, J. and Farver, T. (1986) Xylazine and xylazine-ketamine in dogs. *American Journal of Veterinary Research* **47**:636–641.

Haskins, S., Peiffer, R. and Stowe, R. (1975) A clinical comparison of CT1341, ketamine and xylazine in cats. *American Journal of Veterinary Research* **36(3)**:1537–1543.

Holton, L., Scott, E., Nolan, A., Reid, J., Welsh, E. and Flaherty, D. (1998) Comparison of three methods used for assessment of pain in dogs. *Journal of the American Veterinary Medical Association* **212(1)**:61–66.

Huang, Y., Upton, R. and Rutten, A. (1996) Adverse haemodynamic effects of the rapid intravenous injection of hypotonic solutions in sheep. *Research in Veterinary Science* **60(3)**:209–212.

Hulet, C., Alexander, G. and Hafez, E. *The Behaviour of Sheep*, In *The Behaviour of Domestic Animals* Hafez, E., editor, chapter 11. Baillierre Tindall, London, 3rd edition.

Ihaka, R. and Gentleman, R. (1996) R: A language for data analysis and graphics. *Journal of Computational and Graphical Statistics* **5(3)**:299–314.

Jephcott, E., McMillen, I., Congdon, P. and Thorburn, G. (1988) Electroimmobilisation and ovine plasma cortisol concentrations: effect of current intensity, current duration and diazepam. *Research in Veterinary Science* **44**:21–24.

Jephcott, E., McMillen, I., Rushen, J. and Thorburn, G. (1987) A comparison of the effects of electroimmobilisation and, or, shearing procedures on ovine plasma concentrations of beta-endorphin/beta-lipoprotein and cortisol. *Research In Veterinary Science* **43**:97–100.

Jongman, E., Morris, J., Barnett, J. and Hemsworth, P. (2000) EEG changes in 4-week-old lambs in response to castration, tail docking and mulesing. *Australian Veterinary Journal* **78(5)**:339–343.

Kent, J., Molony, V. and Graham, M. (1998) Comparison of methods for the reduction of acute pain produced by rubber ring castration or tail docking of week-old lambs. *Veterinary Journal* **155(1)**:39–51.

Kent, J., Molony, V. and Robertson, I. (1993) Changes in plasma cortisol concentration in lambs of three ages after three methods of castration and tail docking. *Research in Veterinary Science* **55(2)**:246–251.

Kent, J., Molony, V. and Robertson, I. (1995) Comparison of the burdizzo and rubber ring methods for castrating and tail docking lambs. *Veterinary Record* **136(8)**:192–196.

Kilgour, R. and Dalton, C. (1984) *Livestock Behaviour: A Practical Guide.*. Granada, London.

Klide, A. M., Calderwood, H. W. and Soma, L. R. (1975) Cardiopulmonary effects of xylazine in dogs. *American Journal of Veterinary Research* **36**:931–935.

Kyles, A., Waterman, A. and Livingston, A. (1991) The effects of low dose droperidol on fentanyl analgesia in the sheep. *Acta Veterinaria Scandinavica* **Suppl 87**:176–178.

Kyles, A., Waterman, A. and Livingston, A. (1993a) The spinal antinociceptive activity of the alpha 2-adrenoceptor agonist, xylazine in sheep. *British Journal of Pharmacology* **108(4)**:907–13.

Kyles, A., Waterman, A., Livingston, A. and Vetmed, B. (1993b) Antinociceptive effects of combining low doses of neuroleptic drugs and fentanyl in sheep. *American Journal of Veterinary Research* **54(9)**:1483–1488.

Ley, S., Waterman, A. and Livingston, A. (1990) Variation in the analgesic effects of xylazine in different breeds of sheep. *Veterinary Record* **126**:508.

Ley, S., Waterman, A. and Livingston, A. (1991) The influence of chronic pain on the analgesic effects of the alpha 2-adrenoceptor agonist, xylazine, in sheep. *Journal of Veterinary Pharmacology and Therapeutics* **14(2)**:141–144.

Lineberry, C. G. *Laboratory Animals in Pain Research.*, In *Methods of Animal Experimentation* Gay, W. I., editor. Academic Press, New York.

Livingston, A., Acevedo, M., Kiles, A. and Waterman, A. (1991) The effects of droperidol on fentanyl induced dysphoria in sheep. *Acta Veterinaria Scandinavica* **87**:170–172.

Ludbrook, G., Grant, C., Upton, R. and Penhall, C. (1995) A method for frequent measurement of sedation and analgesia in sheep using the response to a ramped electrical stimulus. *Journal of Pharmacological and Toxicological Methods* **33**:17–22.

Ludbrook, G., Upton, R., Grant, C. and Gray, E. (1996) Cerebral effects of propofol following bolus administration in sheep. *Anaesthesia and Intensive Care* **24(1)**:26–31.

Masal, A., Santa, G., Alagna, S., Anania, V., Frassetto, G., Rovasio, P. and Semiani, A. (1985) Effect of clonidine on stress-induced cortisol release during surgery. *Pharmacological Research Communications* **17**:293–298.

Maze, M. and Tranquilli, W. (1991) Alpha 2-adrenoceptor agonists: defining the role in clinical anesthesia. *Anesthesiology* **74**:581–605.

McNatty, K., Cashmore, M. and Young, A. (1972) Diurnal variation in plasma cortisol levels in sheep. *Journal of Endocrinology* **54**:361–362.

Mellor, D. and Murray, L. (1989a) Changes in the cortisol responses of lambs to tail docking, castration and ACTH injection during the first seven days after birth. *Research in Veterinary Science* **46(3)**:392–395.

Mellor, D. and Murray, L. (1989b) Effects of tail docking and castration on behaviour and plasma cortisol concentrations in young lambs. *Research in Veterinary Science* **46(3)**:387–391.

Mercy, A., Peet, R., Johnson, T., Cousins, D., Robertson, G., Batey, R. and McKenzie, D. (1985) Evaluation of a non surgical technique for sterilising rams. *Australian Veterinary Journal* **62**:350–352.

Molony, V. and Kent, J. (1997) Assessment of acute pain in farm animals using behavioural and physiological measurements. *Journal of Animal Science* **75(1)**:266–272.

Molony, V., Kent, J., Hosie, B. and Graham, M. (1997) Reduction in pain suffered by lambs at castration. *Veterinary Journal* **153**:205–213.

Molony, V., Kent, J. and Robertson, I. (1993) Behavioural responses of lambs of three ages in the first three hours after three methods of castration and tail docking. *Research in Veterinary Science* **55(2)**:236–245.

Morris, J., Ong, R., O'Dwyer, J., Barnett, J., Hemsworth, P., Clarke, I. and Jongman, E. (1997) Pain-related cerebral potentials in response to acute painful electrical stimulation in sheep. *Australian Veterinary Journal* **75(12)**:883–886.

Morton, D. and Griffiths, P. (1985) Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record* **116**:431–436.

Motulsky, H. (1995) *Intuitive Biostatistics*. Oxford University Press, New York.

Muge, D., Chambers, J., Livingston, A. and Waterman, A. (1994) Analgesic effects of medetomidine in sheep. *Veterinary Record* **135(2)**:43–44.

National Health and Medical Research Council (1995) *Australian code of practice for the care and use of animals for scientific purposes..* Australian Government Publishing Service, Canberra.

National Health and Medical Research Council (1998) *Acute pain management scientific evidence.* Ampersand Editorial & Design, Canberra, Australia.

National Research Council (1992) *Recognition and alleviation of pain anddistress in laboratory animals..* National Academy Press, Washington DC.

Nolan, A., Livingston, A., Morris, R. and Waterman, A. (1987a) Techniques for comparison of thermal and mechanical nociceptive stimuli in the sheep. *Journal of Pharmacological Methods* **17**:39–49.

Nolan, A., Livingston, A. and Waterman, A. (1985) The use of a thermal device to assess analgesics in the sheep. *British Journal of Pharmacology* **Supp. 86**:462P.

Nolan, A., Livingston, A. and Waterman, A. (1987b) Investigation of the antinociceptive activity of buprenorphine in sheep. *British Journal of Pharmacology* **92**:527–533.

O'Flynn, M. and O'Dea, J. (1996) The present state of national animal welfare policy. *Australian Veterinary Journal* **73(4)**:121–124.

Ong, R., Morris, J., O'Dwyer, J., Barnett, J., Hemswoth, P. and Clarke, I. (1997) Behavioural and EEG changes in sheep in response to painful acute electrical stimuli. *Australian Veterinary Journal* **75(3)**:189–193.

Riviere, J., Jimenez, T. M., Sundlof, S. and Craigmill, A. (1997) Interspecies allometric analysis of the comparative pharmacokinetics of 44 drugs across veterinary and laboratory animal species. *Journal of Veterinary Pharmacology and Therapeutics* **20(6)**:453–463.

Runciman, W., Ilsley, A., Mather, L., Carapetis, R. and Rao, M. (1984a) A sheep preparation for studying interactions between blood flow and drug disposition. I: Physiological profile. *British Journal of Anaesthesia* **56**:1015–1027.

Runciman, W., Mather, L., Ilsley, A., Carapetis, R. and McLean, C. (1984b) A sheep preparation for studying interactions between blood flow and drug disposition. ii: Experimental applications. *British Journal of Anaesthesia* **56(10)**:1117–1129.

Rushen, J. (1986a) Aversion of sheep to electro-immobilization and physical restraint. *Applied Animal Behaviour Science* **15**:315–324.

Rushen, J. (1986b) Some problems with the physiological concept of stress. *Australian Veterinary Journal* **63(11)**:359–360.

Rushen, J. (1996) Using aversion learning techniques to assess the mental state, suffering, and welfare of farm animals. *Journal of Animal Science* **74(8)**:1990–1995.

Rushen, J. and Congdon, P. (1986) Sheep may be more averse to electro-immobilisation than shearing. *Australian Veterinary Journal* **63(11)**:373–374.

Scott, D. (1976) *Understanding EEG*. Gerald Duckworth and Co., London, UK.

Scott, P., Sargison, N., Strachan, W. and Penny, C. (1996) Assessment of intrathecal lignocaine analgesia for open castration of rams. *British Veterinary Journal* **152(4)**:481–483.

Shutt, D., Fell, L., Connell, R. and Bell, A. (1988a) Stress responses in lambs docked and castrated surgicallly or by the application of rubber rings. *Australian Veterinary Journal* **65**:5–7.

Shutt, D., Fell, L., Connell, R., Bell, A., Wallace, C. and Smith, A. (1987) Stress induced changes in plasma concentrations of immunireactive beta-endorphin and cortisol in response to routine surgical procedures in lambs. *Australian Journal of Biological Science* **40**:97–103.

Shutt, D., Smith, A., Wallace, C., Connell, R. and Fell, L. (1988b) Effect of myiasis and acute restraint stress on plasma levels of immunoreactive beta-endorphin, adrenocorticotrophin (ACTH) and cortisol in the sheep. *Australian Journal of Biological Science* **41(3)**:297–301.

Sorell, G., Hynd, P., Hocking, J., Kuchel, T. and deSaram, W. (1990) The use of high-energy electrons to depilate the breech of sheep. *Australian Veterinary Journal* **67(2)**:51–55.

Steffey, E. and Booth, N. *Local Anaesthetics*, In *Veterinary Pharmacology and Therapeutics* Adams, R. H., editor. Iowa State University Press, Ames, Iowa, Seventh edition.

Stevenson, P. (1994) *For their own good.*. Compassion in World Farming Trust, Petersfield, UK.

Thornton, P. and Waterman-Pearson, A. (1999) Quantification of the pain and distress responses to castration in young lambs. *Research in Veterinary Science* **66**:107–118.

Townsend, C. (1985) *Pulling the Wool.*. Hale and Tremonger.

Tranquilli, W. and Maze, M. (1993) Clinical pharmacology and use of alpha 2-adrenergic agonists in veterinary anaesthesia. *Anaesthetic Pharmacology Review* **1(3)**:297–309.

Wagner, A. E., Muir, W. W. and Hinchcliff, K. W. (1991) Cardiovascular effects of xylazine and detomidine in horses. *American Journal of Veterinary Research* **52(5)**:651–657.

Waterman, A., Livingston, A. and Amin, A. (1991) Analgesic activity and respiratory effects of butorphanol in sheep. *Research in Veterinary Science* **51(1)**:19–23.

Waterman, A., Livingston, A. and Bouchenafa, O. (1988) Analgesic effects of intrathecally-applied alpha 2-adrenoceptor agonists in conscious unrestrained sheep. *Neuropharmacology* **27(2)**:213–216.

Waterman, A., Nolan, A. and Livingston, A. (1987) Influence of idazoxan on the respiratory blood gas changes induced by alpha 2-adrenoceptor agonist drugs in conscious sheep. *Veterinary Record* **121**:105–107.

Watts, J., Murray, M. and Graham, N. (1979) The blowfly strike problem of sheep in New South Wales. *Australian Veterinary Journal* **55**:325–334.

Welsh, E. and Nolan, A. (1995) Effect of flunixin meglumine on the thresholds to mechanical stimulation in healthy and lame sheep. *Research in Veterinary Science* **58(1)**:61–66.

Westhues, M. and Fritsch, R. (1965) *Animal Anaesthesia*. Oliver and Boyd Ltd, Edinburgh.

Wood, G., Molony, V., Walker, S. F., Hodgson, J. and Mellor, D. (1991) Effects of local anaesthesia and intravenous naloxone on the changes in behaviour and plasma concentrations of cortisol produced by castration and tail docking with tight rubber rings in young lambs. *Research in Veterinary Science* **51(2)**:193–199.

Yi, D. and Barr, G. (1996) The suppression of formalin-induced fos expression by different anesthetic agents in the infant rat. *Developmental Psychobiology* **29(6)**:497–506.