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The social function of pain-related behaviour and novel techniques for the assessment of pain in lambs

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Abstract

Pain is a phenomenon that is physical and emotional. There is growing evidence to support the idea that emotional neural systems in the brain drive future behaviour. The behavioural reactions accompanying the pain experience are highly varied, which suggests multiple functions. Pain-related behaviour often doesn't occur in isolation. Instead, it is observable by other animals, thus providing access to information about the emotional state of the animal.

The purpose of this thesis was to investigate pain-related behaviour within a social context. In the simplest terms, animal interactions occur in a dyad, where the animal displaying a particular repertoire of behaviours is the actor and another viewing and potentially responding to the actor's behaviour is the observer. Each individual can be an actor and observer simultaneously.

Domestic sheep are a good model species for studying pain from a social perspective. They are a social species with a strong tendency to form groups. Sheep provide us with an opportunity to evaluate the social influences on pain within the context of painful husbandry procedures normally undertaken in New Zealand such as tail docking, ear tagging and castration. There is also some evidence that the social context affects the behaviour and emotional state of sheep.

The social influences on pain perception and expression have only just begun to be investigated and the studies contained in this thesis add a great deal to this research area. Therefore, the literature review was presented at the end of the thesis and made reference to the findings of the previous experimental chapters of this thesis and introduced a social/communicative function for pain related behaviour.

This other chapters then provided evidence for the social function of pain behaviour, by investigating both sides of the actor-observer dyad. Two chapters focussed on the actor lamb. One, which is presented as two papers, investigated novel ways pain may be expressed by lambs. These are the first studies to demonstrate changes in the ear posture and facial expression of lambs associated with the negative experience of pain. The other chapter, presented as another two papers, investigated what factors affect pain expression by lambs. One study of this chapter demonstrates that the ontogeny of pain processing appears to differ between male and female lambs, and the other demonstrates that expression of pain behaviour depends on the relationship between the actor and observer lamb and previous experience of the test environment.

There is also one chapter, comprised of one paper, which focussed on the observer lamb. This paper investigated how pain expression by the actor lamb affected the behavioural expression of the observer. This study demonstrated socially facilitated behaviour of lambs, possibly indicative of empathy, in response to conspecific pain using quantitative behavioural methods, and a novel qualitative assessment technique.

This thesis culminates in a general discussion chapter which assesses the methodologies used and their limitations, as well as drawing together the research presented in this thesis and analyzing it in the context of the social communicative function of pain.

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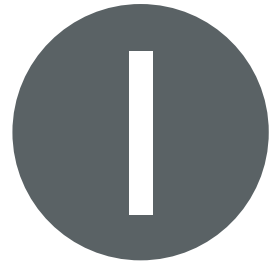
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General Introduction



General Introduction

I

“Being social” means more than just being physically close to others. It means interacting and engaging with other individuals for activities like eating, travel, rest, rearing infants, or mating (Lee, 1994). As humans, our social environment shapes our behaviour to such an extent that we may lie to conform, or exaggerate or suppress our actions in the presence of others (Bond and Smith, 1996; Buck et al., 1992). We do these things because being a social animal has benefits to the individual. As a group, we are more efficient at gathering or producing the resources we need, and better able to share knowledge with others, including our descendents, all of which ultimately benefit the individual (Hamilton, 1971).

However, we are not alone in our sociality. Many other animal species are social in some form or another: be it aggregating together for one activity like hunting or foraging or being part of a group with complex rules and stable relationships for nearly all activities they undertake (Lee, 1994). For other animals, the benefits to the individual of being social include the reduced likelihood of predation, increased foraging efficiency, enhanced thermoregulation, and information sharing (Hamilton, 1971; Jarvis, 1981; Lee, 1994; Trune and Slobodchikoff, 1976).

Being social requires understanding one another in order for animals to live effectively within a social group. Individuals must be aware of, and respond appropriately to, the behaviour of others (Clayton, 1978). In the simplest terms, animal interactions occur in a dyad, where the animal displaying a particular repertoire of behaviours is the actor and another viewing and potentially responding to the actor’s behaviour is the observer. Each individual can be an actor and observer simultaneously. That is, the behaviour of each affects the other. More complex social situations arise when there is more than one actor, or observer. This thesis will examine both sides of the actor-observer dyad in a series of chapters outlined on the next page.

A change in the behaviour of an observer in the presence of an actor is referred to as socially facilitated behaviour (SFB) (Clayton, 1978). SFB is advantageous in a social environment as it acts to synchronize the activity of the group and maintain group cohesion (Clayton, 1978). Empathy is a plausible mechanism underlying SFB. The term ‘empathy’ from a human-focused standpoint refers to the ability to attribute mental states such as desires, beliefs and intentions to another (Singer, 2006). This restricts empathy to a purely human capability (Baron-Cohen et al., 1985; Perner et al., 1987) However many non-human animals show SFB which suggests some degree of empathic capability. Hence, empathy here is defined as the shared emotional and neural state between two individuals, which leads to the generation of an appropriate action (Preston and de Waal, 2002).

How animals respond to one another in a social group (SFB) depends on a number of factors such as whether they are related (Kavaliers et al., 2005; Silk et al., 2005), whether they know the other (Soma and Hasegawa, 2004), the sex of the individuals (Vigil and Coulombe, 2011), their age (Eisenberg et al., 2013), and previous experiences (Stel et al., 2010). Therefore we can't begin to predict behaviour in a social group without considering these factors.

How the social environment influences pain perception and expression is particularly interesting. Pain is highly subjective, and has both physical and emotional aspects (Allen, 2004; Barnett, 1997; Bateson, 1991). More specifically, it is “an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues. It changes the animal's physiology and behaviour” (Molony and Kent, 1997). On the one hand, the experience of pain may be influenced by the external environment, including the social environment (Hamilton, 1964; Kikusui et al., 2006). On the other hand, viewing another individual in pain may have behavioural and emotional effects on the observer (Langford et al., 2006; Langford et al., 2010b; Watanabe and Ono, 1986).

This thesis was born out a desire to understand more about how social animals interact, particularly in relation to pain. A key question I wanted to answer was whether animals can feel empathy for others in pain. From this came a realization that we need less invasive and more sensitive techniques for assessing and understanding pain in animals. Can we use the same or similar methods as we use to explain human pain?

Domestic sheep are a good model species for studying pain from a social perspective. They are a social species with a strong tendency to form groups (Esztevez et al., 2007). Sheep provide us with an opportunity to evaluate the social influences on pain within the context of painful husbandry procedures normally undertaken in New Zealand such as tail docking, ear tagging and castration (Mellor and Stafford, 2000). Because these procedures occur at a young age, lambs were used for the experiments in this thesis. There is also some evidence already existing that the social context affects the emotional state and behaviour of sheep (González et al., 2013; Hild et al., 2010; Ligout and Porter, 2004; Porter et al., 1995). However, the effects of variables such as age, sex, relatedness, and familiarity on pain have not been systematically investigated in this species.

The research reported in this thesis used sheep, specifically lambs, as a model to assess the expression of pain-related behaviour within a social context.

Outline of this thesis

The thesis focuses on three key areas which are explored over five chapters, each of which is a stand-alone paper, with its own introduction, materials and methods, results and discussion. For this reason, there may be some overlap in the methods section of each chapter. The overall findings of the various studies are discussed within the context of previous work in this field. Some of these chapters have already been published and the others are undergoing the review process (indicated at the beginning of each chapter).

A review of the relevant literature is provided at the beginning of each chapter. The overall “literature review” is presented at the end of the thesis in the form of a publishable review paper. The review paper will make reference to the findings of the previous experimental chapters of this thesis because the social influences on pain perception and expression have only just begun to be investigated and the studies contained in this thesis add a great deal to this research area. It is therefore useful to discuss them within the existing literature. This review will also provide a basis for future investigation into the social function of pain-related behaviour by other researchers.

An overview of the three key areas of investigation, and each sub-chapter follows:

1. Novel ways to measure pain expression through ear posture (chapter 2.1) and facial expression (chapter 2.2).

Chapter 2.1 Lambs show ear posture changes when experiencing pain

Ears are essential for obtaining information from the environment (Manteuffel, 2006) but ear posture, or the frequency of postural changes, may also reflect various emotional states of animals. There is evidence that sheep pay attention to, and display (Ferreira et al., 2004; Kendrick et al., 1995; Kendrick et al., 1996; Kendrick et al., 2007; Kendrick et al., 2001), different ear postures according to their emotional experience. This paper demonstrates that lambs in pain spend more time with their ears back, less time with them in a horizontal position, and change their ear posture more often than lambs not experiencing pain.

Chapter 2.2 Coding and quantification of a facial expression for pain by lambs

Ear postures may be part of a wider facial expression for pain. Facial expressions reflecting a variety of emotions can be described in terms of changes to particular facial features (Craig et al., 1992; Prkachin, 1992). Recently, there has been interest in developing coding systems for grimacing in non-human animals such as mice (Langford et al., 2010a), rats (Sotocinal et al., 2011), and rabbits (Keating et al., 2012). The aim of this study was to identify whether lambs produce a noticeable changes in facial expressions when experiencing pain and then to develop a ‘Grimace Scale’ that incorporates these expressions. Human observers were able to consistently distinguish between lambs in pain and those that were not. Furthermore, facial action units showed significant quantitative changes during the experience of pain in lambs. This study is the first to demonstrate

changes in lamb facial expression associated with pain.

2. Factors affecting pain expression, including age and sex (chapter 3.1) and social context and previous experience (chapter 3.2)

Chapter 3.1 The effects of age and sex on pain sensitivity in young lambs

In order for any pain assessment method to be useful, we must consider what factors influence the expression of pain by an actor.

There is evidence that pain sensitivity varies according to both age (Iwata et al., 2002; Jourdan et al., 2000; Pickering et al., 2001; Serrano et al., 2002) and sex (Chesterton et al., 2003; Fillingim and Maxiner, 1995; Mogil et al., 2000; Negus et al., 2004; Riley et al., 1998; Woodrow et al., 1972) and that these factors may also have an interactive effect on pain sensitivity (Beatty and Fessler, 1975). While age effects on behavioural or physiological responses to pain induced by injury have been assessed in lambs, effects on baseline pain sensitivity have not. In addition, the influence of an individual's sex on pain sensitivity have not been evaluated at all in sheep. This study demonstrates that the ontogeny of thermal pain processing appears to differ between male and female lambs.

Chapter 3.2 Social context and other factors influence the behavioural expression of pain by lambs

There are mixed findings regarding the effects of social context on pain expression in sheep. While the presence of the ewe decreased the intensity of pain expression in lambs (Hild et al., 2010), the presence of an unrelated lamb also in pain had no analgesic effect on physiological and behavioural indicators of pain in castrated lambs (Colditz et al., 2012). This suggests that, like in rodents and humans, the relationship between the observer and actor influences the effect of social context on pain expression. However, this has never been specifically tested in any species other than rodents. This is the first study to demonstrate that the occurrence of social buffering on lamb pain behaviour depends on the relationship between the actor and observer and on previous experience of the test environment.

3. How pain expression affects the behaviour and emotional expression of conspecifics (chapter 4)

Previous work on non-human SFB and empathy associated with pain has focused on rodent models, but there is reason to believe that sheep may also experience empathy in such situations. There are mixed findings regarding the effects of social context on the expression of SFB by observers. While ewes responded differently to lambs in pain than to lambs being handled (Edgar et al., 2010), the presence of an unrelated lamb in pain had no effect on the behaviour or physiology of an observer lamb who was also experiencing pain (Colditz et al., 2012).

There are no prescribed descriptor lists for empathic behaviour in lambs. Therefore a qualitative assessment technique may be useful alongside a quantitative ethogram method for evaluating the potential for sheep empathy associated with pain. Qualitative Behavioural Assessment (QBA) is a 'whole animal' approach which integrates many features of the animal's behaviour such as body posture and movement, as well as context, to assess their response to an event or situation (Wemelsfelder et al., 2001). This study demonstrates SFB of twin lambs, possibly indicative of empathy, in response to conspecific pain using quantitative behavioural methods, and QBA.

Each chapter builds on the previous one and culminates in a review paper (Chapter 5) followed by a general discussion chapter (Chapter 6) which assesses the methodologies used and their limitations, as well as drawing together the research presented in this thesis and analyzing it in the context of the social communicative function of pain.

A note on the presentation of P values throughout the thesis: When a significant main or interaction effect was found, a Bonferroni adjustment was made for multiple post hoc comparisons. The P values that are presented are those corrected for multiple comparisons. That is, I have taken the uncorrected P value provided for a particular post hoc comparison and multiplied it by the number of tests, which is the equivalent of a Bonferroni adjustment. Thus a difference was considered significant when the corrected P value was < 0.05.

Novel techniques for assessing pain in lambs



This first chapter presents two novel techniques for assessing pain in lambs: ear posture and facial expression. Both techniques are less invasive than physiological measures and may represent more subtle expressions of pain than whole body behaviours that could serve a social communicative function.

Lambs show ear posture changes when experiencing pain

2.1

This chapter is based on the following paper:

Guesgen, M.J., Beausoleil, N.J., Minot, E.O., Stewart, M., Stafford, K.J. Lambs show ear posture changes when experiencing pain. *Animal Welfare* (under review)

Abstract

Ears are essential for obtaining information from the environment but ear posture, or the frequency of postural changes, may also reflect various emotional states of animals. In adult sheep, the 'forward' ear posture has been associated with negative experiences whereas the 'plane' posture has been associated with positive ones. This study aimed to see whether ear postures related to the experience of pain in lambs and, if so, whether they were influenced by factors such as social environment. The ear behaviour of four to eight week old lambs ($n = 45$) was measured for 30 s, 15 min before and 15 min after tail-docking using a rubber ring. Lambs were tested with another lamb that was either familiar and related (FR), familiar but unrelated (FU) or unfamiliar and unrelated (UU). Each lamb was exposed to the test environment twice, once as the 'actor' (i.e. tail-docked) and once as the 'observer' (not tail-docked). Lambs were docked in one of two rounds, so that half the lambs were docked in their first exposure to the test environment and half were docked in their second exposure two weeks later. Tail-docking was associated with an increase in the proportion of time spent with Ears Backward and a decrease in the proportion of time spent with Ears Plane. There was also a significant increase in the number of changes between ear postures from after docking. There was some evidence that the social context during testing affected ear-related response to docking. Only FR lambs showed an increase in the time spent with ears asymmetrical after docking. This is the first study to demonstrate changes in the ear posture of lambs associated with the negative experience of pain. Ear posture is a non-invasive indicator of lamb physical pain and potential welfare compromise.

Keywords: Animal Welfare; Ear posture; Emotion; Husbandry; Lamb; Pain

Introduction

Ears are essential for obtaining information from the environment (Manteuffel, 2006) but ear posture, or the frequency of postural changes, may also reflect various emotional states of animals. Ear posture may also be purposefully manipulated by an animal to signal status or intent. Therefore, ear posture may be a useful mode of communication as ears are a clearly visible body part (Fox, 1971; Williams, 2002).

There is evidence that sheep pay attention to, and display (Ferreira et al., 2004; Kendrick et al., 1995; Kendrick et al., 1996; Kendrick et al., 2007; Kendrick et al., 2001; Vögeli et al., 2014), different ear postures according to their emotional experience. Vessier et al. (2009) reported a relationship between ear postures and the responses of sheep to their environment according to the suddenness, familiarity, predictability and consistency of events or situations, factors which are thought to underlie a range of emotions. Sheep experiencing a negative emotion, elicited through separation from the flock, displayed a greater number of ear posture changes and spent more time with ears in a forward, or, raised, position (Reefmann et al., 2009a; Reefmann et al., 2009b; Stubsoen et al., 2009). Conversely, situations such as feeding, which were expected to elicit positive emotions, were associated with more 'axial' or 'passive' ear postures (Reefmann et al., 2009a; Reefmann et al., 2009b; Stubsoen et al., 2009). Boissy et al. (2011) went further, to suggest that negative situations can be characterized as either controllable or uncontrollable and that this 'controllability' affected ear posture. Uncontrollable situations (such as inability to control access to food) were associated with ears being backward, whereas controllable situations (ability to access food by passing through a photobeam) were associated with an ears forward posture (Boissy et al., 2011).

There are mixed findings regarding the effects of social environment on the expression of pain-related behaviours in lambs. While the presence of the ewe decreased the intensity of pain expression in lambs (Hild et al., 2010), the presence of an unrelated observer lamb also in pain had no analgesic effect on physiological and behavioural indicators of pain in castrated lambs (Colditz et al., 2012). The expression of pain via ear postures may act as a cue to other group members (Fox, 1971; Williams, 2002), with conspecifics consequently engaging in helping or care behaviour (Hamilton, 1964).

To date no studies have evaluated ear behaviour in response to pain in sheep. Pain, by definition, is a negative emotional experience (Molony & Kent 1997) and tail-docking has been shown to cause pain as indicated by various behavioural (Grant 2004, Mellor & Stafford 2000, Thornton & Waterman-Pearson 2002) and physiological responses (Johnson, et al. 2009, Jongman, et al. 2000, Lester, et al. 1996). Two studies have identified ear posture changes in response to pain in rabbits (Keating, et al. 2012) and horses (Dalla Costa, et al. 2014) as part of a wider facial expression for pain in these species. Both rabbits and horses held their ears backward when in pain.

The aim of this study was to see whether ear postures changed during the experience of pain in lambs and, if so, whether these changes were influenced by factors such as social environment during testing. We hypothesized that the pain associated with tail-docking would result in a change in ear postures as well as the frequency of ear posture changes.

Methods

This study was part of a larger project examining the effects of social environment on the expression of pain in lambs. The results for other pain-related behaviours are reported elsewhere (Chapter 3.2).

Animals and General Care

All procedures were approved by the Massey University Animal Ethics Committee (Protocol 10/24). The study was undertaken at the AgResearch Whatawhata farm in Hamilton, New Zealand in August 2010. Forty-five mixed-age Romney cross ewes and 65 of their single and twin lambs were used in this study. Prior to lambing and in the four weeks prior to testing, the ewes and lambs were kept on pasture according to normal New Zealand husbandry practice.

After birth, ewes and lambs were left undisturbed for at least 3 h to facilitate bonding and suckling. Within the first 24 h after birth, ewe/lamb pairs were brought into a covered area and allocated to one of three treatment groups. Each lamb had a unique identification number sprayed on its back while ewes were identified by their ear tags. Date of birth, ewe tag number, treatment group, sex and whether the lamb was a single or twin was recorded. Ewes and lambs were then moved into one of three new paddocks according to their treatment group (described below); the pairs were kept in these paddocks for four weeks before the first round of testing began. During these four weeks, 15 of the 65 lambs were excluded from the study because one twin in a related pair to be tested together died so the other could no longer be tested ($n = 9$) or because the sex of the lamb had not been recorded ($n = 6$). No animals died during the trial.

Treatment

Fifty lambs were allocated to one of three treatment groups: Familiar Related (FR, $n = 8$ pairs), Familiar Unrelated (FU, $n = 9$ pairs) or Unfamiliar Unrelated (UU, $n = 8$ pairs). These groups reflected the test lamb's relatedness to, and presumed familiarity with, the other lamb with which it was tested at tail-docking (test-mate). FR test-mates were twins (i.e. sibling lambs born on the same day) and therefore were kept in the same paddock with their dam during the four weeks before the first round of testing (i.e. familiar). FU test-mates were kept in the same paddock for four weeks (familiar) but were from different ewes (unrelated). UU test-mates were kept in separate paddocks (unfamiliar) and were unrelated to each other.

Treatment paddocks were similar in size, approximately 80 m x 50 m. Two paddocks (A and C) were adjacent to one another, whereas the other (B) was separated from A and C by a dirt road. The FR and FU groups were kept in paddocks A and C, respectively. Lamb/ewe pairs in the UU group were kept half in paddock A and half in paddock B; UU lambs were tested with a lamb from the other paddock so that there had been no opportunity for close social contact between them before testing.

We attempted to balance lamb sex over the three treatment groups, and to balance the numbers of singletons and twins in the FU and UU groups (Table 1). Because of the large number of twins born, this was not possible and it was necessary to allocate lambs with siblings to the FU and UU groups (Table 1). In these cases, twin pairs were paired for testing with other twin pairs from a different ewe. One lamb from each twin pair was randomly selected to be tested; the other twin from each pair was excluded from testing altogether. During testing the non-tested twins were kept with their dams.

Experimental Set Up

Tail-docking and observations of behaviour were undertaken in a barn with dirt floors. Each lamb was exposed to the test environment twice, once as the 'actor' (i.e. tail-docked) and once as the 'observer' (not tail-docked). Lambs were docked in one of two rounds, so that half the lambs were docked in their first exposure to the test environment and half were docked in their second exposure.

Round one started when lambs were approximately four weeks old and was completed over eight consecutive days. Each day, three to four pairs were tested with at least one pair from each treatment group to minimize any effects of day of testing on behaviour. Round two started six days after the end of round one, when lambs were six weeks old. Pairs were retested in the same order as round one. This ensured that each lamb docked in round one had 14 days to recover from docking before it was exposed to the test environment again. In round two, the lamb that had been the actor in round one became the observer and vice versa.

Testing Procedure

On the day of testing, lambs and dams were brought into the covered area one group at a time. The lambs to be tested and their dams were separated from the rest of the flock and brought, one at a time, into the barn. The remaining animals were returned to the paddock.

In the barn, the two lambs to be tested were separated from their dams and placed together in a 2 m² pen. Their dams were held together in an adjacent 4 m² pen and were provided with food (Fiber Pro: Fiber Fresh Feeds Ltd., Reporoa, New Zealand) and water. The sides of the pens were wooden bars so that the lambs and dams had visual,

olfactory and limited physical access to each other throughout testing.

When FU and UU twins were tested the remaining twin or twins were put in the adjacent pen with their dams.

Once inside the test pen, one of the lambs was randomly selected and a dot was painted on its shoulder to denote that it would be docked (actor). A video camera (Sony Handycam DCR-SR20, Sony Electronics Asia Pacific Pte Ltd.) was set up at the front of the test pen and angled to capture as much of the pen as possible. The lambs were allowed 30 min to settle, undisturbed by human presence, before recording commenced.

After the settling time, undisturbed lamb behaviour was video-recorded for 30 minutes before tail-docking (pre-docking period). One researcher then entered the pen and restrained the actor lamb to allow another experimenter to apply the docking ring. The rubber ring was applied using an elastrator between two tail vertebrae at a point allowing sufficient tail proximal to the ring to cover the anus (and vulva for female lambs). When the ring had been applied, the researchers left the pen, and lamb behaviour was recorded for a further 30 minutes (post-docking period). Lambs and dams were then released from the pens and returned to their allocated paddock.

Analysis of Actor Lambs' Ear Behaviour

The ear behaviour of actor lambs was scored for 30 s, halfway through each recording period (i.e. at 15 min before docking and at 15 min after docking). The post-docking sample was taken 15 min after docking as this is when the peak frequency of other pain-related behaviours occurs when rubber rings are used (Lester et al., 1996). The pre-docking sample was taken 15 min into the undisturbed recording period for consistency. A sample duration of 30 s was chosen based on previous studies looking at ear posture (Boissy et al., 2011; Reefmann et al., 2009a; Reefmann et al., 2012; Reefmann et al., 2009b; Stubsjoen et al., 2009; Veissier et al., 2009).

The time spent with ears in each of four positions was scored according to Table 2 (Visual examples provided in Figure 1), as was the number of changes among ear positions (Ear Change frequency). Some actor lambs spent a small proportion of the sampling time out of view of the camera. Therefore the proportion of the total in-view time the lamb spent with its ears in each position was calculated and analyzed. Five out of 50 actor lambs were out of view for the whole duration of one of the video recordings. This meant that their data for both the pre- and post-docking periods were excluded from the analysis (final $n=45$, Table 1).

Table 1: The number of male, female, singleton and twin lambs in each treatment group (FR= Familiar/related, FU= Familiar/unrelated, UU= Unfamiliar/unrelated) included in the analysis. This number excludes 5 lambs that were out of camera view for the entire 30 second sampling period.

Treatment group	Sex	Singletons	Twins	TOTAL
FR	M	0	6	6
	F	0	7	7
FU	M	4	3	7
	F	7	2	9
UU	M	6	3	9
	F	5	2	7
TOTAL		22	23	45

Table 2: Ear-related behaviours scored for actor lambs, based on Reefman et al. (2009a) and Veissier et al. (2009). State behaviours are mutually exclusive.

Behaviour	Description
State behaviours	
Ears Plane	Both ears are perpendicular to the head-rump axis. This is often also associated with the ear auricle facing down.
Ears Forward	Both ears are positioned forward of the perpendicular. This is often also associated with the ear auricles facing forward.
Ears Backward	Both ears are positioned behind the perpendicular. The ear auricles are not visible from the front.
Ears Asymmetrical	The left and right ears are positioned differently from one another, in one of the other three postures described above.
Event behaviours	
Ear change	The number of times ear position changed from one of the above to another

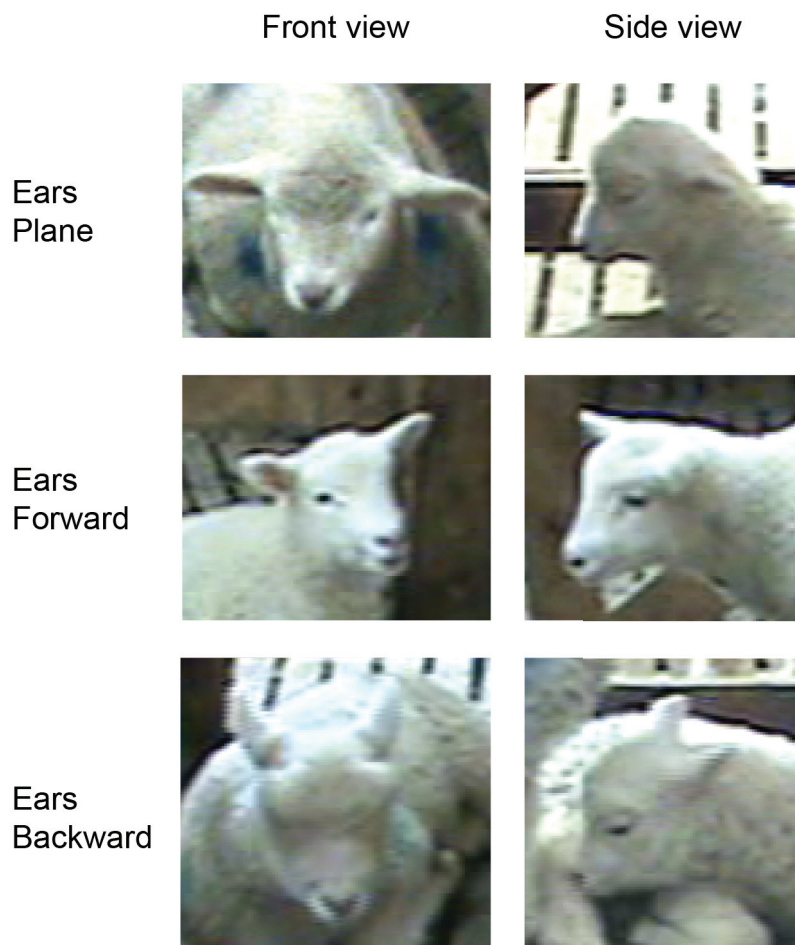


Figure 1: Visual examples of the ear postures scored.

Statistical Analysis

All statistical analyses were performed using SAS Version 9.2 (SAS Institute Inc., North Carolina, USA). Pre/post data could not be transformed to satisfy assumptions for parametric analyses, therefore repeated measure analyses were performed on ranked data. A MIXED model was used to evaluate the effects of tail-docking on ear-related behaviour with period (pre- and post-docking) as the repeated measure, lamb as the random effect, and treatment (FR, FU, UU), round (1, 2) and pair-sex (same-sex pair both female, same-sex pair both male, mixed pair actor female, mixed pair actor male) as fixed effects. When significant effects were found, post hoc tests, corrected for multiple comparisons using a Bonferroni adjustment, were performed. Differences were considered significant at $P < 0.05$.

Results

There was a significant effect of period on all behaviours except time spent with ears forward (Table 3).

Tail-docking was associated with an increase in the proportion of time spent with Ears Backward and a decrease in the proportion of time spent with Ears Plane (median \pm interquartile range: Backward: pre 0.0 ± 0.1 , post 0.6 ± 0.4 ; Plane: pre 0.6 ± 0.7 , post 0.1 ± 0.3). There was also a significant increase in the number of changes between ear postures from pre- to post-docking (median \pm interquartile range: pre 5 ± 6 , post 9 ± 6).

There was an interactive effect of period and treatment on time spent with Ears Asymmetrical (Table 3). FR lambs spent more time with ears asymmetrical after docking (Figure 2), whereas there was no significant change in this posture for the other treatment groups. There were no significant differences among treatment groups before or after docking.

No other significant effects or their interaction were found (Table 3).

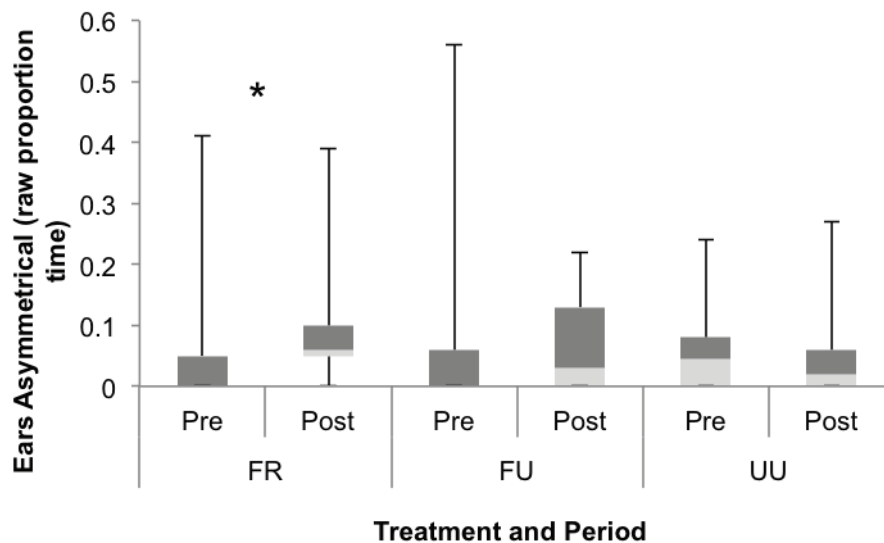


Figure 2: Median \pm interquartile range proportion of time with Ears Asymmetrical before and after tail docking. FR= Familiar Related, FU= Familiar Unrelated, UU= Unfamiliar Unrelated. Significant differences at $P < 0.05$ are denoted by an asterisk (*).

Table 3: Results of MIXED model analysis on frequency or duration of actors' behaviour before and after tail-docking (period). Factors included in the model are pair-sex (same-sex pair both female, same-sex pair both male, mixed-pair actor female, mixed-pair actor male), round (1, 2) and treatment (Familiar Related, Familiar Unrelated, Unfamiliar Unrelated). Bold text denotes a statistically significant result at $P < 0.05$.

Behaviour	Period		Pair-Sex		Round		Treatment		Significant Interactive Effects		
	$F_{(1,29)}$	p	$F_{(3,26)}$	p	$F_{(1,26)}$	p	$F_{(2,26)}$	p	Type	$F_{(df)}$	p
Ears Plane	31.35	<0.0001	0.48	0.70	0.39	0.54	0.11	0.89			
Ears Forward	0.05	0.83	1.36	0.28	3.48	0.07	1.16	0.33			
Ears Backward	55.97	<0.0001	0.18	0.91	1.22	0.28	0.17	0.85			
Ears Asymmetrical	5.73	0.02	1.76	0.18	1.85	0.19	1.00	0.38	Period*Treatment	4.73 _(2,29)	0.02
Ear Changes	24.26	<0.0001	1.81	0.17	1.13	0.30	1.24	0.31			

Discussion

This study investigated whether, and how, ear posture changes in lambs when they are experiencing physical pain. We found there was a significant effect of tail-docking on ear behaviour. The same lambs also showed significant changes in other pain-related behaviours such as increases in active behaviours and time spent in abnormal postures and decrease in time spent in normal postures after docking (Chapter 3.2). Therefore, we can infer that any concurrent change in ear posture post-docking is related to the experience of pain in lambs.

Lambs spent significantly less time with their ears plane, more time with their ears backwards and showed a greater frequency of ear postural changes after docking. However, the time spent with ears forward did not change significantly as a result of docking. In accordance with previous research, we found that a negative experience, in this case significant pain due to tail docking, was associated with a decrease in the time spent with ears in a plane position. This posture has previously been associated with emotionally positive situations, such as rumination or standing in the feed area (Boissy et al., 2011; Reefmann et al., 2009a; Reefmann et al., 2009b). Therefore, it is likely that Ears Plane reflects the absence of strong negative emotions as was likely in the pre-docking period after lambs had time to settle.

In the present study tail-docking was also associated with an increase in time spent with ears backward. Our findings are consistent with some studies in other species such as horses and rabbits where pain elicited backward ear postures (Dalla Costa, et al. 2014, Keating, et al. 2012) as well as with studies of silver foxes and cattle, where negative situations elicited backward ear postures (Coulon, et al. 2011, Moe, et al. 2006). They also agree with findings that the controllability, or lack thereof, of a negative situation can influence ear posture. The cause of pain is likely perceived to be an uncontrollable situation by lambs, which may explain the increase in the backward ear posture (Boissy, et al. 2011).

While our findings are similar in some respects to those of previous studies investigating ear posture changes, there are some key differences. Several previous studies interpreted the forward ear posture as indicative of negative emotion, elicited by separation from other group members (Reefmann et al., 2009a; Reefmann et al., 2009b; Stubsgjoen et al., 2009). Separation from the flock will elicit increased alertness or arousal as the sheep tries to reunite with the group. While this may be accompanied by negative emotion, ears forward is more likely a behavioural strategy used to rectify the problem of social separation. Lambs in our study were not visually separated from their dams and could make some physical contact. Therefore, no changes in ears forward were found.

Consistent with previous studies on negative emotions, the negative experience of tail-docking was also associated with a significant increase in the number of ear posture changes (Reefmann et al., 2009a; Reefmann et al., 2012; Reefmann et al., 2009b). This

increase may reflect the conflicting motivations of the animal. After docking, the lamb was expressing its pain by holding its ears in a backward posture; however it would still be motivated to remain alert to its surrounding environment. Hence, the frequency of change between these postures could represent the strength of the motivation to meet both the requirements of alertness and pain expression. Another interpretation of these results is that an increase in ear posture changes due to docking reflects anxiety. In this and previous studies using separation from the flock as a negative stimulus it is difficult to disentangle pain, fear, and anxiety as these emotions often occur concurrently.

We also sought to investigate the effects of social environment, particularly the familiarity and relatedness of other lambs in the test environment, on ear postures. There was some evidence that the social environment during testing affected the ear behaviour due to docking. Only lambs tested with a familiar twin showed a significant increase in the time spent with ears asymmetrical as a result of docking. This is consistent with the finding that lambs tested with a twin showed a smaller change in some other pain-related behaviours than lambs tested with familiar, but unrelated, test-mates (Chapter 3.2). In previous studies, the asymmetrical ear posture was associated with social separation (Reefmann et al., 2012) as well as with a sudden, unexpected event (Boissy et al., 2011) suggesting it might be indicative of a negative emotional experience. In the present study, the small amount of time spent with ears in this posture suggests it likely represented a transitional posture when ears were moved from one posture to another. This may indicate the desire to simultaneously attend to more than one relevant stimulus in the environment. In twin lamb pairs, the twin that is observing may represent a more relevant stimulus than a lamb that is unrelated or unfamiliar because of the greater likelihood that they will engage in helping behaviour (Hamilton, 1964). This is consistent with the idea that lambs may alter their ear posture to indicate their emotional state to other group members.

In order to investigate the conclusions of this study further, it might be useful to do a cross-fostering experiment. Twin lambs will spend more time together than unrelated lambs in the same paddock, so it is unclear whether it is the genetic component of being a twin or the greater familiarity that facilitates social buffering in twin lambs. A cross-fostering experiment, in which twin lambs are reared apart or unrelated lambs are reared together, would help tease apart these effects. In addition, it might be interesting to investigate whether the degree of pain is related to the frequency of ear posture changes, or time spent holding a particular ear posture.

Animal Welfare, and other, implications

Ear postures appear to have a different function to other pain-related behaviours that may function to assist in alleviating pain (for example an increase in abnormal lying) (Allen, 2004).

The expression of pain via ear postures may act as a cue to other group members (Fox, 1971; Williams, 2002). Conspecifics may consequently engage in helping or care behaviour. Sending out, and paying attention to, signals or cues of a conspecific may be particularly useful in a social species, such as sheep, as it can enhance survival (Clayton, 1978; Maynard Smith and Harper, 2003). As well as providing information to conspecifics, ear postures provide a useful cue for welfare assessment. In addition to pain brought on by tail-docking or other husbandry procedures, ear posture could be used as a non-invasive indicator of pain due to other diseases, for example foot rot.

Conclusion

This is the first study to demonstrate changes in the ear posture of lambs associated with the negative experience of pain. The results also suggest that there may be an effect of social context on lamb ear postures in response to tail-docking. These findings indicate that ear behaviour, and pain-related behaviour in general, may have a social function. The possibility of using ear posture as a welfare indicator is an avenue for further investigation.

Scoring and quantification of a facial expression for pain in lambs

2.2

This chapter is based on the following paper:

M.J. Guesgen, N.J. Beausoleil, E.O. Minot, M. Stewart, Matthew Leach, K.J. Stafford. Scoring and quantification of a facial expression for pain in lambs. Behavioural Processes (in preparation).

Abstract

Facial expressions are routinely used to assess pain in humans, particularly those who are non-verbal. Recently, there has been an interest in developing coding systems for facial grimacing in non-human animals, such as rodents and rabbits. These grimace scales have been shown to be accurate, reliable and valid measures of pain in these species. The aim of this preliminary study was to identify whether lambs produce noticeable changes in facial expressions when experiencing pain and then to develop a 'Grimace Scale' that incorporates these expressions. By comparing images of lambs before (no pain) and after (pain) tail-docking, the Lamb Grimace Scale (LGS) was devised in consultation with scientists experienced in assessing facial expression in other species. The LGS consists of five facial action units: Orbital Tightening, Mouth Features, Nose Features, Cheek Flattening and Ear Posture. In Experiment I, still images of the face of seven restrained lambs were taken from video footage before and after tail-docking or sham tail docking from the both front and side angle (total 16 images per lamb). These images were scored by five naïve human observers using a Lamb Grimace Scale (LGS). For each image, observers scored each facial action unit with a value of 0 (action unit absent), 1 (action unit moderately present), 2 (action unit obviously present), or 9 (don't know) as well as giving an overall pain score. Data were analyzed as two separate datasets for stills taken from the front and side. For each facial action unit and the overall pain score a linear MIXED model with period, treatment, and observer as fixed effects and lamb number as the repeated measures subject and treatment as the group. An intraclass correlation coefficient (ICC) was used to assess how consistently observers scored each still for a particular action unit. In Experiment II, video recordings of the face of unrestrained, as well as restrained, lambs ($n = 9$) were taken before and after tail-docking. Still images from this footage were scored by a different group of five observers using the LGS in the same way as Experiment I, with each facial action unit also being quantified by a researcher using image measurement software. Consistency of scoring using the LGS was overall good ($ICC = 0.7$) but was lower when looking at the lamb from the side and for the feature of Cheek Flattening.

Furthermore, there was little agreement between experiments as to which features observers scored differently after docking. In Experiment I Nose Features, Mouth Features, Overall Pain Score changed after docking while in Experiment II Orbital Tightening and Ear Posture. In addition, the changes in observer scores for experiment II did not match up with the features that changed quantitatively (Observer scores: Orbital Tightening, Ear Posture; Quantitative: Mouth angle, eye aperture right). This may indicate one of two things, either that there is no consistently recognized facial expression for pain in lambs, or that limitations of the methodology meant we the experimenters did not find more consistency between experiments. This study is the first to demonstrate changes in lamb facial expression associated with pain. We encourage future research to verify the results presented here.

Keywords: Facial expression; Grimace; Lamb; Pain

Introduction

Most mammalian species can change their face in response to a range of stimuli or experiences (Diogo et al., 2009). Mammalian facial expressions may serve an adaptive function, whereby information about emotion, intent or the environment can be sent to a nearby observer (Waller and Micheletta, 2013). In humans, describing facial expression is nearly synonymous with describing emotion (Waller and Micheletta, 2013). Facial expressions reflecting a variety of emotions can be described in terms of changes to particular facial features. Several studies identify a specific facial expression, or grimace, for pain in humans. This can be described in terms of brow lowering, cheek raise, eyelid tightening, nose wrinkle and eye closing (Craig et al., 1992; Prkachin, 1992). Identifying human pain via facial grimace is useful, as it allows clinicians to assess pain in non-verbal patients (Hicks et al., 2001).

Recently, there has been interest in developing coding systems for grimacing in non-human animals. A Mouse Grimace Scale (MGS) was developed by compiling photographs of mice before and after a standard 0.9% acetic acid abdominal constriction test and coding which facial features changed when the animal was in pain (Langford et al., 2010). The MGS consists of five key action units: orbital tightening, nose bulge, cheek bulge, ear position and whisker change. A Rat Grimace Scale (RGS) was developed in a similar way (Sotocinal et al., 2011). The action units of the RGS are broadly similar to those of the MGS, however pain results in cheek flattening in rats as opposed to bulging in mice. The Rabbit Grimace Scale (RbtGS) used ear tattooing as the pain stimulus to identify similar action units to those identified in both the mouse and rat scales: orbital tightening, cheek flattening, nose shape, whisker position and ear position (Keating et al., 2012). Finally, the Horse Grimace Scale (HGS) was developed using routine castration and identified six facial action units: stiffly backward ears, orbital tightening, tension above the eye area, prominent strained chewing muscles, mouth strained and pronounced chin, and strained nostrils and flattening of the profile (Dalla Costa et al., 2014)

All three scales demonstrated a high inter-rater reliability and accuracy. In addition, the MGS has been used to assess the efficacy of four common post-operative analgesics as well as post-operative pain from vasectomy (Leach et al., 2012; Matsumiya et al., 2012), the RbtGS has been used to evaluate the efficacy of topical analgesic EMLA cream (Keating et al., 2012), and the HGS has been used to assess horses undergoing routine castration (Dalla Costa et al., 2014)

Sheep may also show a noticeable change in facial expression due to pain. Domestic sheep experience pain routinely as they undergo painful husbandry procedures including ear tagging, tail docking and castration (Mellor and Stafford, 2000). They are also a social species with a strong tendency to form groups (Esztevez et al., 2007) and are diurnal, therefore that any change in facial expression may be recognized and responded to by other members of the group. Taken together, this may indicate a communicative function of facial expression in a social species such as sheep (Williams, 2002).

The aims of this study were to firstly investigate whether lambs produce a noticeable facial expression when experiencing pain and then to develop a grimace scale that incorporates any such expressions. We then undertook two experiments to assess the usefulness of this scoring scale. The first, evaluated the effects of tail docking on human observers' perceptions of lamb facial features as well as the consistency in scoring across human observers using the same dataset that was used to generate the LGS. The second, evaluated the effects of both tail-docking and restraint on both human observers' perceptions of lamb facial features, and on quantitative measures of facial features.

Methods

The Massey University Animal Ethics Committee approved all procedures for both experiments (Protocol 12/104).

Experiment I: Tail docking versus sham tail docking

Animals

Nine 5 to 6 week old Romney cross lambs were used in this study (n female = 4, n male = 5). Lambs were randomly selected from a flock of 40 lambs and their dams. This study was undertaken at the Massey University Keebles Farm in Palmerston North, New Zealand. Prior to testing, the ewes and lambs were kept on pasture according to normal husbandry practice. Testing was undertaken in an outdoor yard with concrete floors.

Experimental Procedure

On the day of testing, the dams and lambs were brought in from the paddock as a flock and kept in a holding yard. One lamb at a time was randomly selected for testing. The same person picked the lambs up and held them in a seated position for the duration of observation. All lambs were tested over one day.

Lambs were alternately allocated to one of two treatments both applied by the farm manager. They were either tail-docked using a rubber ring or sham-docked (control). The rubber ring was applied using an elastrator between two tail vertebrae at a point allowing sufficient tail proximal to the ring to cover the anus (and vulva for female lambs). In the sham docking the farm manager handled the tail area for 15 s, to replicate docking without the ring being applied.

For 1 minute before (pre) and 10 minutes after (post) treatment the lamb's face was recorded using a high definition video camera (Sony Handycam DCR-SR20, Sony Electronics Asia Pacific Pte Ltd., Tokyo, Japan). Two different angles, front and side, were captured with two cameras.

Frame Capture

Two lambs were excluded from the study as one had received an ear notch prior to filming, which may have altered its pain perception and one fell asleep during the sham docking procedure and hence its facial expression may have been incorrectly interpreted (Langford et al., 2010; Sotocinal et al., 2011) (final lamb n = 7: 3 control (1 female), 4 docked (2 female)).

For each of the remaining lambs, four images were extracted manually from the video recordings for each angle (front, side) and each period (pre- and post-treatment) to produce 16 still images per lamb. Stills were selected from across the 1 min pre-docking period and in the last 5 min of the post-treatment period, as lambs show a high frequency of other pain-related behaviours around this time after docking (Molony and Kent, 1997); thus it was a sensible time to look for pain-related facial feature changes. Images were selected at regular intervals across the minute and 5 minutes (that is, approximately every 15 seconds in the pre- and every 75 seconds in the post-period), however because the lamb may have been moving at that particular moment, this was not always possible. Individual frames were "grabbed" using screen capture and cropped using Preview (Apple Inc., California, USA) so that the body and most of the background was no longer visible. All of the resulting 112 images were used for development of the grimace scale and subsequently for scoring by human observers.

LGS Development

The Lamb Grimace Scale (LGS) was developed using methods similar to those used to develop the rat, mouse and rabbit scales (Keating et al., 2012; Langford et al., 2010; Sotocinal et al., 2011). By comparing images of the four docked lambs before (no pain) and after (pain) docking, the LGS was devised in consultation with scientists experienced in assessing facial expression in other species. Action units were selected on the basis of their presence and consistency across lambs in pain. The LGS consists of five facial action units (Table 4): Orbital Tightening, Nose Features, Mouth Features, Cheek Flattening, and Ear Posture. As with the rat, mouse, and rabbit scales, all facial action units were unweighted.

Table 4: Description of the Lamb Grimace Scale action units. Note that Ear Posture were not scored in Experiment I.

Action Unit	Description
Orbital Tightening	Lambs in pain show “squeezing” of the eye or closing of the eye, described as orbital tightening. This may only occur, or occur more strongly, in one eye. If the eye closure reduces the visibility of the eye by more than half, it would be scored as severe (2) on a scale from 0-2.
Nose Features	The nose of lambs in pain appears tightened with a decrease in nostril size. Tightening may be depicted through flattening or ‘pointing’. Flattening makes the nose appear more like a horizontal line in frontal headshots, whereas pointing makes the nose appear more ‘V’ rather than ‘U’ shaped in frontal headshots.
Mouth Features	The lips of a lamb in pain are flattened and tightened. The lips appear more like a horizontal line in frontal headshots. There is lack of the ‘upwards curl’ at the edge of the lips that gives lambs their ‘smiling’ appearance when not in pain.
Cheek Flattening	Lambs in pain show less bulging of the nose and cheek area. In severe cases, the cheek has a ‘hollowed’ appearance. When not in pain, the cheeks appear rounded in frontal headshots.
Ear Posture	Lambs in pain have ears that are tense and point backwards or downwards so that you cannot see the inner part of the ear. As a result, ears may appear narrower, and flattened dorsally. When lambs are not in pain, their ears are relaxed and horizontal, or slightly forward of the head and you can see the inner ear. Note, please use discretion when scoring lambs where the ear posture is obscured by leaning against objects, e.g. pen wall.

Lamb Grimace Scale Scoring

Image files were numbered and their order randomized. Each image was then copied into Excel (Microsoft Corporation, Redmond, Washington) with one image per tab.

The group of human observers consisted of three postgraduate students, one animal welfare scientist and one professor of laboratory and companion animal welfare. All had experience observing animals including sheep, however none, except the professor, were familiar with grimace scale scoring.

Human observers were given two files: A detailed instruction sheet, explaining the action units and providing image examples and the Excel scoring spreadsheet containing 112 images. Participants were blind to the treatment of the lambs. For each image, participants were asked to score a value of 0, 1, 2 or 9 for each of the five LGS action units (excluding ear posture) (Table 4). Ear Posture was not scored in this experiment as the restraint applied to the lamb may have confounded ear posture scores. A score of zero indicated confidence by the scorer that the action unit was absent. One indicated confidence by the scorer that the action unit was moderately present. Two indicated confidence by the scorer that the action unit was obviously present.

Nine indicated the scorer didn't know how, or did not feel confident assigning a score to a particular action unit for this still. Participants were also asked to give an overall pain score on the same scale, based on their general impression. This was done after they had scored the other facial features for that particular still.

Statistical Analyses

Analyses were performed using SAS Version 9.2 (SAS Institute Inc., North Carolina, USA). When one or two stills (out of a possible four) were scored as 'don't know' (9), a value was imputed from an average of the other three, or two, stills. The scores for the four stills were averaged to give one score per lamb, per period, per angle, per action unit. Cases where there were more than two stills scored as 'don't know' were treated as missing values.

Residuals were generated and tested for normality of distribution. The data were also tested for homogeneity of variance between periods. If the assumptions for robust parametric statistical methods were met, analyses were performed on raw data. If not, data were transformed using Blom's normalized ranks before analysis.

Data were analyzed as two separate datasets for stills taken from the front and side. For each facial action unit and the overall pain score a linear MIXED model was used to evaluate the effects of tail docking on observers' perception of facial feature changes with period (pre-, post-docking), treatment (docked, control) and observer (observers 1-5) as fixed effects, and lamb number (1-7) as the repeated measures subject and treatment as the group. Sex could not be included in the analysis because of low lamb numbers.

Consistency analyses were performed using SPSS v. 19 (SPSS Inc.). The intraclass correlation coefficient (ICC) was used to assess how consistently observers scored each still for a particular action unit. ICCs were calculated separately for front and side angles, with each dataset being split into three groups: Pre-period (both control and docked lambs), post- period (docked) and post- period (control).

Experiment II: Tail docking when restrained/not restrained

Animals

Nine 5 to 6 week old Romney cross lambs were used in this study (n female = 4, n male = 5, different from those used in Experiment I). This study was undertaken at the Massey University Keebles farm in Palmerston North, New Zealand. Prior to testing, the sheep were kept on pasture. Testing was undertaken in a semi-covered outdoor yard with concrete floors.

Preparation Procedure

Dams and their lambs were brought in from the paddock as a flock and kept in a holding yard. The lambs to be tested were selected from the flock and placed in a pre-testing pen approximately 3 m². The remaining lambs and flock were then returned to the paddock. Each test lamb was caught and restrained while the researcher placed twelve 1 cm² sticker markers at specific locations on its face as well as a 30 mm by 36 mm rectangular calibration sticker, secured to the center of its forehead. The calibration sticker identified each lamb by a number and was later used for calibrating images for analysis (relating the number of pixels in an image to an actual length in mm). All markers were made from Tiki-Tape™ multi purpose cloth tape (Tiki-Tape N.Z. Ltd., Wellington). The sex and ear tag number of each lamb was recorded and the lamb was then put back in the pre-testing pen with the other test lambs.

Testing Procedure

Testing began 5 min after all the lambs had undergone the preparation procedure, to allow the lambs to settle into the novel environment. Lambs were recorded in four stages, outlined in more detail below: Pre-docking pen (PrP); pre-docking held (PrH); post-docking held (PH); post-docking pen (PP) (Figure 3).

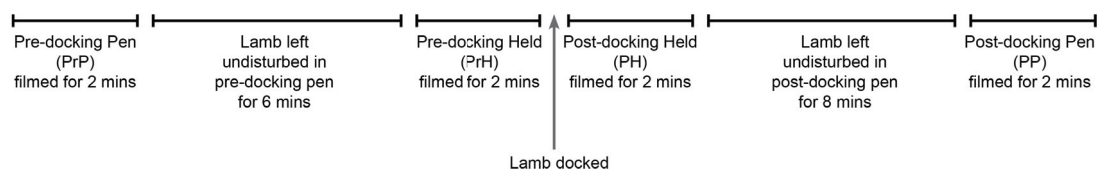


Figure 3: Timeline of experimental procedure for Experiment II: Tail docking when restrained/not restrained.

In the PrP stage, each lamb was video recorded for 2 min in the pre-testing pen. The experimenters filmed from outside the pen to minimize disturbance to the lambs. Each lamb was then caught and restrained by Experimenter II in the same order in which they had been filmed previously, 6 min after the end of filming in the PrP stage. Experimenter I then filmed the lamb's face for a further 2 min in the PrH stage. This was to ensure that the start time for filming in the pre-docking pen was 10 min before docking, to correspond with the start time for filming in the post-docking pen. Experimenter I then docked the lamb using a rubber ring and recorded the time of docking. The docking procedure was carried out in the same way as in Experiment I. The lamb was then filmed 2 min for another 2 min after this application while still restrained. This was the PH stage. Subsequently, the lamb was put in a post-testing pen, adjacent to the pre-testing pen and of similar size. Ten min after the lamb was docked (that is, 8 minutes after the end of the PH stage), the lamb was filmed for 2 min in the post-testing pen (PP stage). Again, the lambs were filmed from outside the pen. Lambs were filmed in the same order in which they were docked, so that the time between docking and filming was consistent for all lambs. Lambs served as their own controls in this experiment, so all lambs were tail-docked.

All recording of the lambs was done using a high definition video camera (Sony Handycam DCR-SR20, Sony Electronics Asia Pacific Pte Ltd., Tokyo, Japan). All videos were recorded from a front-on angle, providing a close-up of the lamb's face. The experimenter followed the lamb from outside the pen and the camera was moved by the experimenter with the lamb's face to avoid unintentional changes in facial features due to the movement of the head laterally.

Frame Capture

For each of the nine lambs, three images were extracted manually from each of the four videos (PrP, PrH, PH, PP) to produce 12 still images per lamb (total $n = 108$). Individual frames were "grabbed" using screen capture and cropped using Preview (Apple Inc., California, USA) so that the body and most of the background was no longer visible. Stills were selected across the 2-min videos for the PrP, PrH and PH periods. For the PP period, stills were taken when the lamb was displaying body behaviour indicative of pain, as has been previously validated by Molony and Kent (1997). These behaviours were either: neck arch, lateral lying or stamp. This was not done for the PH period, as the movement of the lamb was restricted.

Two lambs did not appear to show any of these body behaviours in which case stills were taken at regular intervals across the 2-min PP period.

Measurement of facial feature changes

Five agriculture or animal science postgraduate students (different from those who participated in Experiment I) scored the 108 images according to the procedure described in the section ‘Lamb Grimace Scale Scoring’ (Appendix 1). All had experience observing animals including sheep, however none were familiar with grimace scale scoring.

Image files were also scored quantitatively using ImageJ software (National Institutes of Health, <http://rsbweb.nih.gov/ij/index.html>) by Mirjam Guesgen. Each image was first calibrated by drawing a line that was the length of the calibration sticker and assigning the known length of 36 mm to the length in pixels of that line. This allowed measurements taken across different images to be compared directly. Six facial features were measured according to Table 5. An example image is provided (Figure 4). In some cases the lamb was positioned in such a way that it was not possible to measure a particular feature, for example leaning against a pen wall. In these cases, a missing value was recorded.

Table 5: Descriptions of how measurements of the facial features were made in ImageJ for Experiment II: Tail docking when restrained/not restrained

Feature Name	Description	Letter on example image
Eye Aperture Left	Height of the lamb’s left eye, measure in the center of the eye vertically.	A
Eye Aperture Right	Height of the lamb’s right eye, measured in the center of the eye vertically.	(not shown)
Nose Angle	The angle formed when the three sticker markers on the lateral tips of the nose and the center of the nose are joined by a line. The inside corners of the stickers were used as a guide.	B
Mouth Angle	The angle formed when the three sticker markers on the outside corners of the mouth and the sticker on the chin are joined by a line. The inside corners of the stickers were used as a guide.	C
Ear Angle Left	The angle from a perpendicular line to the lamb’s head where the sticker marker was placed to the other sticker marker on the tip of the lamb’s left ear. The inside corner of the sticker on the ear tip was used as a guide.	D
Ear Angle Right	The angle from a perpendicular line to the lamb’s head where the sticker marker was placed to the other sticker marker on the tip of the lamb’s left ear. The inside corner of the sticker on the ear tip was used as a guide.	(not shown)

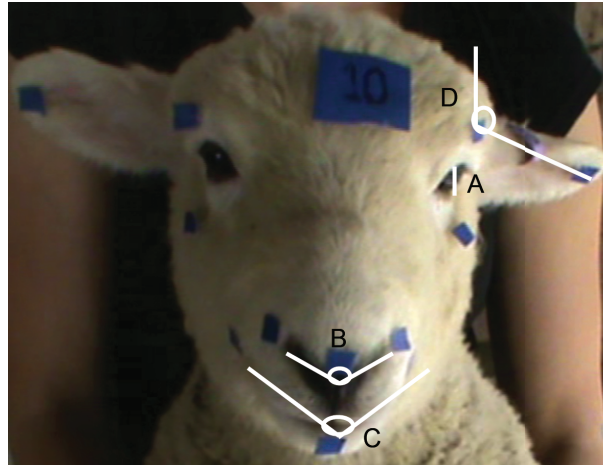


Figure 4: Example image outlining how the facial features of each lamb were measured quantitatively including placement of sticker markers on the test lambs. The larger rectangle denotes a calibration sticker of a known size for later analysis.

Statistical Analyses

Analyses were performed using SAS Version 9.2 (SAS Institute Inc., North Carolina, USA).

For stills where a ‘don’t know’ score was given, a value was imputed from an average of the other two stills for each observer. The scores for the three stills were averaged to give one score per lamb, per period, per action unit. Cases where there was more than one still (out of a possible three) scored as ‘don’t know’ were treated as missing values.

Residuals were generated and tested for normality of distribution. The data were also tested for homogeneity of variance between periods. If the assumptions for robust parametric statistical methods were met, analyses were performed on raw data. If not, data were transformed using Blom’s normalized ranks before analysis.

For each facial action unit and the overall pain score a linear MIXED model was used to evaluate the effects of tail docking and restraint on observers’ perception of facial feature changes (that is, LGS scores for each action unit), with period (pre-, post-docking), restraint (held, pen) and observer (observers 1-5) as fixed effects, and lamb number (1-9) as the repeated measures subject.

Another linear MIXED model was used to evaluate the effects of tail docking on quantitative changes in facial features, with period and restraint as fixed effects and lamb number (1-9) as the repeated measures subject.

Consistency analyses were performed using SPSS v. 19 (SPSS Inc.). The intraclass correlation coefficient (ICC) was used to assess how consistently observers scored each still for a particular action unit. ICCs were calculated separately for each period (pre/pen, pre/held, post/held, post/pen).

Results

Experiment I

Regardless of treatment, there was a significant increase in observers' scores for Orbital Tightening (Period effect: raw mean \pm SE, front: pre 0.8 ± 0.1 , post 1.2 ± 0.1 ; side: pre 1.1 ± 0.1 , post 1.5 ± 0.1) and the Overall Pain Score from the pre to the post treatment period, from both the front and side angles (Period effect: raw mean \pm SE, front: pre 0.8 ± 0.1 , post 1.2 ± 0.1 ; side: pre 1.2 ± 0.1 , post 1.5 ± 0.1). Similarly, there was an increase in observers' scores for Cheek Flattening from the pre to post-treatment period for stills taken from the front (raw mean \pm SE, pre 0.7 ± 0.1 , post 0.9 ± 0.1); and an increase in scores for Nose Features and Mouth Features for stills taken from the side (raw mean \pm SE, Nose features: pre 0.8 ± 0.1 , post 1.2 ± 0.1 ; mean of ranks \pm SE, Mouth features: pre 1.4 ± 0.1 , post 1.6 ± 0.1).

Period and treatment had an interactive effect on observers' scores of Nose Features, Mouth Features and the overall pain score for stills taken from the front (Table 6). Observers scored these facial features significantly higher after docking than before docking for docked lambs but there was no change for control lambs (Figure 5). There were no significant differences between docked and control lambs in the pre- or post-docking periods.

Treatment had an effect on Cheek Flattening scores from the side over both periods (Table 6), with docked lambs scored higher than control lambs overall (mean of ranks \pm SE, docked 1.1 ± 0.04 , control 0.9 ± 0.06). While there was a numerical increase in scores for docked lambs from before to after docking, which likely contributed to this effect, the difference in scores was not significant

Observer had a significant effect on LGS scores for all action units from both the front and side (Table 6). The effect varied, however observers one and five generally scored the lambs more conservatively (lower LGS scores) than the other observers.

Consistency among observers varied according to angle of the still, facial feature and period (Table 7). Observers scored more facial features with 'don't know' when viewing stills taken from the side angle. Similarly, observers often scored Cheek Flattening with 'don't know'. Cheek Flattening also had the lowest consistency between observers, except for in the post-docking period. However, this period also had the highest number of don't know scores. Observers' scores were more consistent after tail-docking than before.

Table 6: Results of linear MIXED model on Lamb Grimace Scale scores generated by five observers in Experiment I: Tail docking versus sham tail docking, before and after tail docking (period) from the front angle and side angle. Factors included in the model are treatment (docked, control) and observer (human observers 1-5). Bold text denotes a statistically significant result at $P < 0.05$.

Front Angle	Facial Feature		Period		Treatment		Observer		Period*Treatment	
	F _(1,5)	p	F _(1,5)	p	F _(1,5)	p	F _(4,24)	p	F _(1,5)	p
	Orbital Tightening	39.14	0.002	0.15	0.717	3.19	0.031	0.21	0.669	
	Nose Features	27.09	0.001	0.15	0.717	5.20	0.004	34.72	0.002	
	Mouth Features	27.09	0.003	0.55	0.490	5.07	0.004	20.29	0.006	
	Cheek Flattening	7.04	0.045	0.27	0.625	2.97	0.040	0.99	0.370	
	Overall Pain Score	35.05	0.002	0.36	0.577	6.92	<0.001	8.84	0.031	

Side Angle	Facial Feature		Period		Treatment		Observer		Period*Treatment	
	F _(1,5)	p	F _(1,5)	p	F _(1,5)	p	F _(4,24)	p	F _(1,5)	p
	Orbital Tightening	11.99	0.020	0.03	0.870	2.98	0.040	0.32	0.600	
	Nose Features	57.50	<0.001	1.51	0.274	10.76	<0.001	0.10	0.770	
	Mouth Features	25.86	0.004	0.06	0.810	4.09	0.011	4.48	0.088	
	Cheek Flattening	0.91	0.380	9.27	0.030	8.59*	<0.001	0.55	0.490	
	Overall Pain Score	9.79	0.026	0.00	0.950	11.90	<0.001	0.26	0.630	

*df = 4, 22 due to 8 missing observations

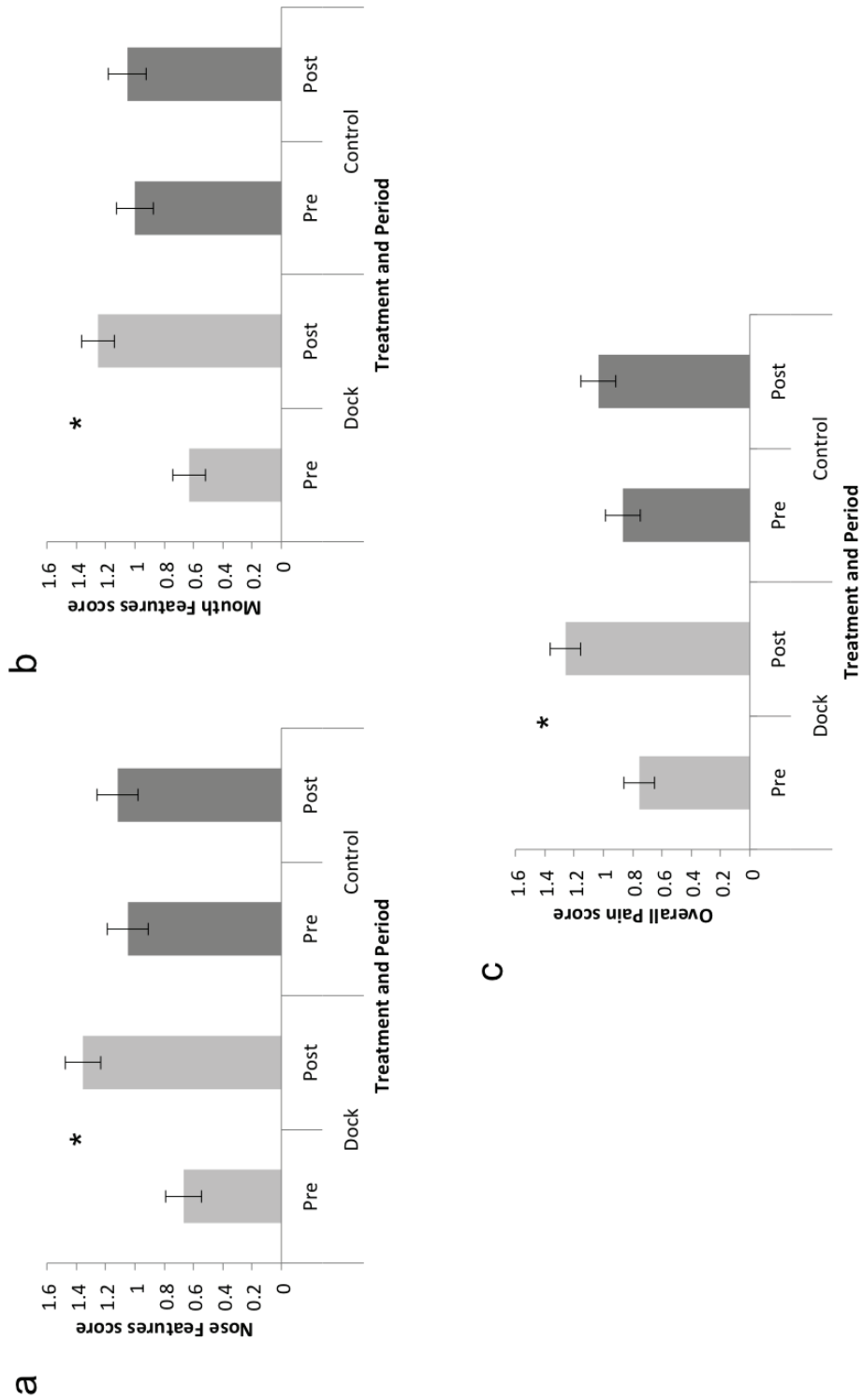


Figure 5: Raw mean \pm SE Lamb Grimace Scale scores of Nose Features (a), Mouth Features (b) and Overall Pain Score (c) from the front angle in Experiment I: Tail docking versus sham tail docking. For each facial action unit and the overall pain score a linear MIXED model was used to evaluate the effects of tail docking and restraint on observers' perception of facial feature changes (that is, LGS scores for each action unit), with period (pre-, post-docking), restraint (held, pen) and observer (observers 1-5) as fixed effects, and lamb number (1-9) as the repeated measures subject. Significant differences at $P < 0.05$ are denoted by an asterisk (*).

Table 7: Intra-class correlation coefficients for scores from five observers for stills of docked and control lambs taken from the front angle and side angle in Experiment I: Tail docking versus sham docking, before and after treatment. N=7 lambs for pre-treatment period, N=4 lambs for post-dock period, N=3 lambs for post-control treatment period.

Front angle	Period	Treatment	Action Unit	ICC	No. don't knows
	Pre	Control/Dock		Orbit	0.72
	Nose			0.80	0
	Mouth			0.64	0
	Cheek			0.44	2
	Pain			0.52	2
Post	Dock		Orbit	0.85	0
			Nose	0.87	0
			Mouth	0.80	0
			Cheek	0.72	6
			Pain	0.78	0
Post	Control		Orbit	0.80	0
			Nose	0.87	0
			Mouth	0.65	0
			Cheek	0.52	0
			Pain	0.78	0

Side angle	Period	Treatment	Action Unit	ICC	No. don't knows
	Pre	Control/Dock		Orbit	0.92
	Nose			0.88	6
	Mouth			0.81	1
	Cheek			0.60	22
	Pain			0.84	5
Post	Dock		Orbit	0.94	2
			Nose	0.88	4
			Mouth	0.83	0
			Cheek	0.95	23
			Pain	0.92	1
Post	Control		Orbit	0.52	0
			Nose	0.11	7
			Mouth	0.39	1
			Cheek	0.13	7
			Pain	0.66	2

Experiment II

Observer scores

Tail docking resulted in a significant increase in observers' scores of Orbital Tightening and Ear Posture (Period effect: Raw mean \pm SE, Orbital Tightening: pre 0.4 ± 0.1 , post 0.5 ± 0.1 ; Ear Posture: pre 0.9 ± 0.1 , post 1.1 ± 0.1).

Restraint also had a significant effect on Orbital Tightening and Ear Posture over both periods (Table 8). When lambs were in the pen observers scored these facial units higher than when lambs were held (Raw mean \pm SE, Orbital Tightening: pen 0.5 ± 0.1 , held 0.3 ± 0.1 ; Ear Posture: pen 1.2 ± 0.1 , held 0.8 ± 0.1).

Which observer viewed the stills had a significant effect on scores for all action units. The effect varied, however observer three generally scored the lambs more conservatively (lower LGS scores) than the other observers and observer two gave higher scores.

Consistency among observers varied according to facial feature and whether the lamb was restrained (Table 9). Observers gave more 'don't know' scores for Mouth Features and Cheek Flattening. The consistency among observers for the Cheek Flattening feature was also lower than other features. The fewest 'don't knows' were for Ear Posture, and observers were also more consistent in scoring this feature than the others. Restraint influenced consistency, with there being less consistency among observers when the lamb was held as opposed to being in the pen.

Quantitative scores

Tail docking resulted in a significant increase in mouth angle and a significant decrease in eye aperture for the right eye (Period effect: Mean of ranks \pm SE, Mouth Angle: pre -0.4 ± 0.2 , post 0.4 ± 0.2 ; Raw mean \pm SE, Eye Aperture Right: pre $12 \text{ mm} \pm 0.7$, post $11 \text{ mm} \pm 0.7$).

Restraint significantly affected the aperture of the right eye, nose angle and the ear angle for both ears (Table 10). When lambs were in the pen they had a significantly smaller eye aperture, nose angle and ear angles than when they were held (Mean of ranks \pm SE, Nose Angle: pen -0.4 ± 0.2 , held 0.4 ± 0.2 ; Ear Angle left: pen -0.3 ± 0.2 , held 0.3 ± 0.3 ; Raw mean \pm SE, Eye Aperture Right: pen $11 \text{ mm} \pm 0.7$, held $12 \text{ mm} \pm 0.7$; Ear Angle right: pen $53^\circ \pm 8.6$, held $87^\circ \pm 8.7$).

Table 8: Results of linear MIXED model on Lamb Grimace Scale scores generated by five observers in Experiment II: Tail docking when restrained/not restrained, before and after tail docking (period),. Factors included in the model are restraint (held, free in pen) and observer (human observers 1-5). Bold text denotes a statistically significant result at $P < 0.05$.

Facial Feature	Period		Restraint		Observer	
	$F_{(4,8)}$	p	$F_{(1,8)}$	p	$F_{(4,32)}$	p
Orbital Tightening	6.52	0.034	12.37	0.007	19.20	<0.001
Nose Features	0.63	0.450	5.06	0.055	9.42	<0.001
Mouth Features	0.00	0.960	0.29	0.610	7.81	<0.001
Cheek Flattening	0.68	0.430	4.87	0.060	8.26	<0.001
Ear Posture	8.94	0.017	61.76	<0.001	1.59	0.199
Overall Pain Score	0.35	0.570	3.54	0.100	4.64	0.005

Table 9: Intra-class correlation coefficients among five observers for stills taken in Experiment II: Tail docking when restrained/not restrained before and after docking and with lambs restrained or in the pen.

Period	Restraint	Action Unit	ICC	No. don't knows
Pre	Pen	Orbit	0.72	2
		Nose	0.63	17
		Mouth	0.86	38
		Cheek	0.85	6
		Ear	0.83	1
		Pain	0.80	2
Pre	Held	Orbit	0.51	0
		Nose	0.80	6
		Mouth	0.37	15
		Cheek	0.39	10
		Ear	0.86	0
		Pain	0.84	0
Post	Held	Orbit	0.47	0
		Nose	0.75	2
		Mouth	0.72	4
		Cheek	0.63	6
		Ear	0.87	1
		Pain	0.74	0
Post	Pen	Orbit	0.81	0
		Nose	0.75	11
		Mouth	0.48	15
		Cheek	0.66	10
		Ear	0.89	2
		Pain	0.75	2

Table 10: Results of linear MIXED model on quantitative measures of lamb facial expression in Experiment II: Tail docking when restrained/not restrained before and after tail docking (period). The other factor included in the model is restraint (held, free in pen). Bold text denotes a statistically significant result at $P < 0.05$.

Facial Feature	Period		Restraint		Period*Restraint	
	$F_{(1,8)}$	p	$F_{(1,8)}$	p	$F_{(1,8)}$	p
Eye Aperture left	2.80	0.13	0.11	0.75	0.12	0.74
Eye Aperture right	6.14	0.04	5.30	0.05	0.22	0.06
Nose Angle	0.58	0.50	10.98	0.01	0.19	0.68
Mouth Angle	8.58	0.02	4.50	0.07	0.02	0.90
Ear Angle left	0.32	0.60	7.68*	0.03	0.96	0.36
Ear Angle right	0.03	0.87	17.13	0.003	1.32	0.28

*df = 1, 7 due to 4 missing observations

Discussion

In this study, we report the development and testing of the Lamb Grimace Scale (LGS), a method to evaluate pain in the lamb using facial features. Docking is painful experience for lambs (Fitzpatrick et al., 2006; Grant, 2004; Mellor and Stafford, 2004; Molony and Kent, 1997), so we can be confident that any change in facial expression after docking relates to pain.

Accuracy and validity

In order for the LGS to be a useful measure of pain, observers must be able to detect differences in facial features according to whether the lamb is experiencing pain. In both experiments, LGS scores for certain facial features increased from before to after docking when lambs underwent a docking treatment. In previous studies examining facial expression in non-human animals, accuracy was assessed by comparing the overall pain score to the *a priori* knowledge of whether the animal was in pain. By doing so, they were able to calculate a percentage of correct identifications of pain/not pain ranging from 73.3% (Dalla Costa et al., 2014) to 83.6% (Keating et al., 2012). However, this measure of accuracy is based on the assumption that all animals were showing facial feature changes. In this study not all lambs showed overt behavioural (and therefore possibly also facial) changes in response to pain. Therefore, any such accuracy measures would have to have been based on a decision by the researchers as to whether a lamb was displaying a grimace.

The validity of the observers' scores can be verified to some extent by measuring changes in facial features quantitatively. The action units to show quantitative changes due to docking were mouth angle and aperture of the right eye. These changes agree with the description of Mouth Features and Orbital Tightening in the LGS i.e. docked lambs had a greater mouth angle, so their mouth looked more like a horizontal line from the front angle. In addition, the aperture of the right eye was smaller, indicating squinting of the eye. While observers did not score a significant change in mouth features after docking in Experiment II, a different set of observers scored a significant flattening in mouth features only in docked lambs after docking in Experiment I. It would also be useful to validate the LGS using other methods such as behaviour (Dalla Costa et al., 2014, Keating et al., 2012), physiology (Keating et al., 2012), or drug-induced analgesia (Sotocinal et al., 2011).

However, there was little agreement between experiments as to which features observers scored differently after docking. In Experiment I Nose Features, Mouth Features, and Overall pain changed after docking, while in Experiment II Orbital Tightening, and Ear Posture changed. In addition, the changes in observer scores for Experiment II did not match up with the features that changed quantitatively. Observers noted Orbital Tightening, and Ear Posture while quantitative measures indicated changes in Mouth angle, and eye aperture. Furthermore, observers in Experiment I perceived all facial features to change with time when the lamb was restrained, even when the lamb had not undergone a docking treatment (period effects). This may indicate one of two things, either that there is no consistently recognized facial expression for pain in lambs, or that limitations of the methodology meant we did not see more consistency between experiments.

Reliability

A higher correlation (greater consistency) among observers would indicate that they are scoring similarly, and that the LGS is useful. Overall, consistency among observers across all facial features, periods, and treatments was fairly good (averaging around 0.70). However this is lower than previous studies which reported 0.90 (Sotocinal et al., 2011), 0.91 (Keating et al., 2012) and 0.92 (Dalla Costa et al., 2014). Consistency of scoring using the LGS differed depending on angle the still image was taken from and the facial action unit scored. Generally, agreement among observers was lower when they were looking at a lamb face from the side (Experiment I). This may be due to asymmetries in facial expression from the left and right sides where we only presented images from the left side of the lamb face (Ekman et al., 2007; Nagasawa et al., 2013) or simply because people are only getting half the visual information of a face from a side view. We would therefore recommend that still images are taken from the front angle.

Some facial action units were scored more, or less, consistently than others. In particular, observers did not agree on their scoring of Cheek Flattening. This feature also had the highest number of 'don't know' scores in Experiment I. This may be because the hollowing of the cheeks was difficult to see from the front angle. It may also be that perceptions of cheek flattening are largely affected by lighting. For example, a light source from above may make the cheeks look more hollow, however if lit from the side, the flattening would be difficult to see. Interestingly, Mouth Features also had low agreement among scorers in Experiment II, but only when lambs were in the pen after docking. Factors such as image quality or angle that the image was taken from may have contributed to this. Observers (who were blind to the treatment) were consistent in their general impression of whether the lamb was in pain, except for in Experiment I before docking (front angle) and when scoring controls after treatment. This may be because observers were primed to be looking for pain so some may have been more likely to attribute pain, even when lambs weren't experiencing it.

Effects of methods

We may not have seen consistent changes in facial features for a number of reasons, such as low lamb numbers, some lambs not showing body behavioural signs of pain at the time points the stills were taken, inconsistencies in observer training, and differences in camera angle. Two out of nine docked lambs in Experiment II did not show behavioural signs that they were in pain. However, it has been observed previously that not all individuals display overt or active behavioural signs of pain after docking (Petrie et al., 1995) and so this finding is perhaps not surprising. These particular lambs may have been displaying a reactive coping style to pain, which is characterized by immobility and passivity (Koolhaas et al., 1999). The values for their facial expression data did not fall outside two standard deviations of the group means so they were not deemed outliers. Thus, they may have been as likely as active copers to have shown facial expressions of pain.

While observer was included in the statistical model to take account of this factor when examining the effects of the main factors of interest, period, restraint and treatment, we none-the-less found a significant effect of observer on all the action features scored, over both experiments. While there were no consistent trends, observer one in Experiment I tended to be more conservative (that is, scored the stills lower overall). This observer was experienced with scoring facial action units in laboratory animals, which may account for this difference. The other observers were fairly equal in the amount of experience they have had with sheep and none had experience scoring facial features, yet observer three generally scored the lambs more conservatively and observer two gave higher scores overall. This indicates that individual differences, possibly personal pain tolerance or ability to empathize with the animals, may influence how observers use the LGS. Observers were sent the scale and asked to familiarize themselves with it before beginning scoring. This process was not supervised, therefore we are unable to know how well observers learnt to use the scale before scoring. Because of this, we would recommend getting a group of observers with similar experience and training all observers together on the LGS before commencing scoring.

There may be little consensus between quantitative and qualitative measures because of asymmetry in lamb facial expression and difficulties accounting for depth. It is likely that lamb facial expression is asymmetrical, meaning that one eye or ear may not show significant change after docking. This may account for the fact that we only found a period effect for the right eye aperture. Lateralization of facial expression, particularly eye and ear movements, has been demonstrated in humans, other primates and dogs (Nagasawa et al., 2013; Rogers and Andrew, 2002). Second, quantitative measures, particularly ear angle, are affected by depth perception more so than qualitative ones. Observers are able to perceive depth within a photograph and can thus tell whether the ears, for example, are pointed backwards or are horizontal. In contrast, when an angle is superimposed on top of a two-dimensional photograph, the depth information is lost.

Both LGS scores and quantitative measures of facial features were influenced by restraint. When lambs were held in Experiment II, they were scored higher on Orbital Tightening and Ear Posture than when they were in the pen. Restraint also significantly affected the angle of the nose and ear angle for both ears when measured quantitatively. Finally, LGS scores for Orbital Tightening and Cheek Flattening from the front angle in Experiment I were significantly higher in the post-docking period overall, even though about half these lambs did not undergo the docking treatment. In Experiment I, lambs were restrained for the entirety of filming (about 13 minutes total including application of the treatment). It may be that lamb facial expression changes as a result of prolonged restraint and this change was misinterpreted as pain by observers. Restraint may also physically limit facial expression, so that the lamb is unable to express a pain face. For example, ear posture is limited by having the lamb lean against the experimenter so they are unable to fully put their ears back. We would therefore suggest that lambs are unrestrained when measuring facial expression due to the effect restraint can have on expression and its interpretation.

It was difficult to maintain the same camera angle as the lamb was moving in the pen. This may have affected observers' interpretation of particular facial features or altered the quantitative measurements of those features. Ideally, we would use some kind of head-mounted system to capture sheep facial expressions so that the camera angle was maintained. The feasibility of such technology is an avenue for further investigation.

The results of these experiments should be interpreted with caution due to low lamb numbers. However, we encourage others to investigate lamb, and sheep, facial expression further. Future studies should increase the number of lambs used, as well as including a control group if they were to replicate Experiment II. In this study, as in previous studies of animal facial expression, facial action units were unweighted. However, it may be interesting in future to assess how much each particular feature is contributing to observer's impression of lamb pain.

Conclusion

This study is the first to demonstrate changes in lamb facial expression associated with pain. Human observers were able to use some facial features to distinguish between lambs in pain and those not. Furthermore, we found significant quantitative changes in some facial action units. However, there was little agreement between experiments as to which features observers scored differently after docking and the changes in observer scores for Experiment II did not correlate well with the features that changed quantitatively. We encourage future research to elucidate whether this means there is no consistently recognizable facial expression for pain in lambs, or that limitations of this methodology meant we did not see more consistency between experiments.

Factors affecting pain expression by actor lambs



I have presented two novel techniques for measuring pain in lambs. However, as the previous chapters have shown, factors such as the relationship between the actor and observer or restraint can influence the expression of pain by actor lambs. This chapter expands on this idea to look at how the factors of age, sex, social context, and previous experience of the test environment affect pain expression.

The effects of age and sex on pain sensitivity in young lambs

3.1

This chapter is based on the following paper:

Guesgen, M.J., Beausoleil, N.J., Minot, E.O., Stewart, M., Stafford, K.J. (2011). The effects of age and sex on pain sensitivity in young lambs. *Applied Animal Behaviour Science* 135, 51-56.

Abstract

Lambs routinely undergo painful husbandry procedures including ear tagging, tail docking and castration. Pain early in life is important because it can compromise lamb welfare. Studies of altricial species, such as rodents and humans, suggest that baseline pain sensitivity varies with age and sex. While age effects on behavioural or physiological responses to pain induced by injury have been assessed in lambs, the effects of age and sex on baseline pain sensitivity have not. The aim of this study was to investigate the effects of age and sex on baseline pain sensitivity in lambs using a remote laser thermal stimulus. Thermal nociceptive thresholds were measured in male and female lambs aged between one and 12 days old. There was no main effect of sex or age on lambs' latency to respond to the thermal stimulus. However, there was a significant interaction effect of sex and age, with male and female pain sensitivity diverging with increasing age. The latency to respond increased with age in males but did not change significantly with age in females. This suggests that older males were less sensitive to thermal pain than younger males. The ontogeny of pain processing appears to differ between male and female lambs. This may be explained by sex-specific changes in peripheral components of the pain processing system (e.g. population, distribution or sensitivity of receptors) or in central integration of nociceptive signals. Alternatively, there may be sex-specific changes with age in other physiological processes relevant to thermal nociception, e.g. peripheral thermoregulation. Future research should look beyond 12 days of age to see whether sex differences in pain sensitivity persist, thus providing a more complete picture of the ontogeny of pain processing development in precocial species.

Keywords: Pain, lamb, age, sex, laser

Introduction

Lambs routinely undergo painful husbandry procedures including ear tagging, tail docking and castration (Mellor and Stafford, 2000). Pain early in life is important because it can compromise lamb welfare (Bateson, 1991). Many countries, including New Zealand, have regulations to reduce animal welfare compromise associated with painful husbandry procedures (MAF, 2005). One way to reduce such compromise may be to determine the most appropriate age at which to perform such procedures.

Pain sensitivity is defined as the point at which nociception, or the ability to sense noxious stimuli, occurs (Allen, 2004). Noxious stimuli are those which actually, or have the potential to, cause damage to living tissue (Woolf and Ma, 2007). In mammals, pain sensitivity can vary according to a number of factors including individual differences, experience, feeding, tactile stimulation, environment, stress, age and sex (Anand et al., 2000; Barrowman et al., 1954; Carbajal et al., 2003; Dodd, 2005; Molony et al., 2002; Negus et al., 2004; Sternberg and Ridgway, 2003).

Studies of altricial mammals (e.g. rodents and humans) suggest that pain sensitivity varies with age. However, there is contradictory information about the effects of age on pain sensitivity, with some studies finding that pain sensitivity increased (Iwata et al., 2002), while others found it decreased (Pickering et al., 2001) or showed no change with age (Jourdan et al., 2000; Serrano et al., 2002). The pain sensitivity of altricial species may be influenced by external stimuli and experience after birth due to the fact they are born in a relatively immature developmental state (Wood et al., 2003). This may account for changes in sensitivity with post-natal age as well as variable reports of age effects on pain sensitivity.

In contrast, precocial species, such as sheep, are born with relatively mature nervous systems and probably undergo less nervous system development post-natally (Wood et al., 2003). Therefore, post-natal age may not have an effect on pain sensitivity in these species and we would expect any differences in pain sensitivity for precocial animals to be present at birth.. However, there is some evidence that older lambs are more sensitive to pain than younger ones. Older lambs show higher frequencies of some pain-related behaviours in response to injury than do younger lambs, suggesting that they find these experiences more painful (Molony et al., 1993; Thornton and Waterman-Pearson, 2002). In addition, older lambs show a more pronounced cerebrocortical response to castration than do very young lambs (Johnson et al., 2009). This supports the idea that pain sensitivity may vary with post-natal age in precocial farm species such as sheep.

While age effects on behavioural or physiological responses to pain induced by injury have been assessed in lambs, effects on baseline pain sensitivity have not. Measuring pain sensitivity through injury is problematic, as inflammation due to injury alters how nociceptive information is processed (Fitzpatrick et al., 2006). In addition, younger lambs may have a lower capacity to express pain-related behaviour than older lambs and therefore appear to show fewer signs of pain (Barrowman et al., 1954; Molony et al., 1993). Finally there may be variation in the extent or nature of tissue damage associated with castration and docking at different ages due to variation in body size (Marai and Bahgat, 2003). Therefore it is important to evaluate age effects on baseline pain sensitivity in lambs.

In addition to age effects, pain sensitivity in mammals has been found to differ between sexes. In adult mammals of various species, it has been demonstrated that adult males are less sensitive to pain than females (Chesterton et al., 2003; Fillingim and Maxiner, 1995; Mogil et al., 2000; Negus et al., 2004; Riley et al., 1998; Woodrow et al., 1972). The factors of sex and age have also been shown to have an interactive effect on pain sensitivity. In rats, sex differences in pain sensitivity did not emerge until after 50 days of age (Beatty and Fessler, 1975). Once again, these results are mostly from studies of altricial species and the effects of sex on pain sensitivity in precocial farm animals have not yet been evaluated.

Pain sensitivity can be evaluated by measuring nociceptive thresholds (Le Bars et al., 2001). Measurement of thermal nociceptive thresholds using a laser produces highly replicable results, at least in cattle (Rushen et al., 1999; Schwartzkopf-Genswein et al., 1998; Veissier et al., 2000). This study investigates the effects of age and sex on baseline pain sensitivity of lambs using a laser thermal stimulus. We hypothesized that older lambs would be more sensitive to pain than younger lambs and that female lambs would be more sensitive to pain than male lambs.

Materials and Methods

Experimental Facilities

This study was undertaken at AgResearch Whatawhata farm, Hamilton, New Zealand. All procedures used in this experiment were approved by Massey University Animal Ethics Committee (Protocol 10/24). The paddock in which the ewes were kept in the days prior to lambing was 500 m from the barn where the lambs were tested. The barn had slatted-floor pens and natural lighting. After lambing, dams and lambs were kept in one of three paddocks (100 m by 50 m) approximately 200 m from the barn.

Animals and General Care

Seventy-five Coopworth cross lambs were used in this study; their dams were of mixed age. Ewes were scanned at mid-pregnancy to choose a group with similar lambing dates. Ewes were pasture-fed prior to lambing. After lambing they were fed on grass and approximately 4400 g Fiber Pro (Fiber Fresh Feeds Ltd., Reporoa, New Zealand) per ewe per day and provided with water *ad libitum*. The same people that undertook the experimental treatments also carried out the daily care and feeding of the animals.

After birth, lambs were left with their dams for at least three hours to allow maternal bonding and suckling. Only single and twin lambs from non-assisted births were used in the study. After allowing time for bonding, lambs and dams were moved to a covered area next to the barn and randomly allocated to their test groups. The majority of lambs were born overnight, therefore the lamb/dam pairs were brought into the covered area in the morning. Each lamb had an identification number sprayed on its back using coloured sheep spray. Date of birth, age at testing (test group), ewe tag number, paddock number, sex and whether the lamb was a single or twin were recorded. Dams and lambs were then moved to their allocated paddock. The farm manager inspected the lambs and dams every few days to ensure the health of the animals.

Experimental Procedure

General Procedure

Within 24 h of birth, each lamb was randomly allocated to one of six test groups corresponding to the age at which it would be tested (one, three, five, seven, nine or 12 days old). Each lamb was tested at only one age. There were at least ten lambs in each group (Table 11). Lambs born on the same day were allocated to different testing groups (for example if six lambs were born, one would be allocated to each test group). We attempted to balance groups for sex, however, because unequal numbers of lambs were born each day and twins were allocated to the same test group, this was not always possible.

The number of lambs tested each day varied between two and 11, with an average of four per day over an 18 day period. Each day there was a mix of test ages to reduce any effects of day of testing on pain sensitivity e.g. not all day three lambs were tested on the same day.

Table 11: Number of male and female lambs tested on each day of age.

Age	Males	Females	TOTAL
1	8	5	13
3	6	9	15
5	5	7	12
7	8	6	14
9	6	5	11
12	5	5	10
TOTAL	38	37	75

On the day of testing, lambs and dams were brought into the covered area one paddock at a time and the lambs to be tested and their dams were separated from the rest of the flock. The remaining animals were returned to the paddock. The lambs to be tested and their dams were then brought, one at a time, into the barn.

In the barn, each lamb/dam pair was held in a separate adjacent pen, approximately 2 m² in size. Fiber Pro and water were provided for the dam. Once the pairs were in place, each lamb was caught and restrained to shave a section of hair approximately 5 cm² from the lateral metatarsus on both hind limbs. Hair was shaved uniformly and no obvious signs of trauma or inflammation were observed. In the case of twins, both lambs were shaved and one lamb was marked with a dot on the hindquarters to differentiate the lambs and to ensure that each lamb was only tested the required number of times. The lambs were then allowed two hours to settle with their dams. During the settling period the laser device was set up and the researchers moved quietly around the barn, thus allowing the lambs and dams to become accustomed to the environment and their presence.

Testing Procedure

After the settling time, three threshold tests were conducted on each lamb with at least a 15 min interval between each (T1, T2, T3). Testing occurred in the pen with the dam. Lambs were tested in the order in which they were brought into the barn and twins were tested one after another. Testing did not commence until the lamb was in the appropriate testing position (sternal recumbency with one or both hind limbs extended). This sometimes resulted in longer than 15 min between tests.

The laser was supported on a tripod approximately 1.5 m in front of the lambs and operated remotely to reduce the effects of human presence on the lamb's attention and behaviour. When the lamb was in the appropriate position, the laser was aimed at the shaved area of one leg and turned on. A timer, which was part of the laser device but was operated by the researcher, was started when the LED light denoting that the laser was functional turned on. The laser was left running until the animal responded by withdrawing its leg, at which time it was turned off and the time was recorded to the nearest 0.1 s. The device was programmed to automatically turn off after 15 s, even if the lamb had not responded. This was to avoid tissue damage to the animal (Schwartzkopf-Genswein, personal communication).

The temperature that elicited a reflex behavioural response (withdrawal of the limb) was considered to represent the lamb's thermal nociceptive threshold. In this study, because the skin temperature was assumed to be increasing constantly over time (Veissier et al., 2000), we measured the latency to respond rather than the actual skin temperature. As soon as the threshold was reached, the laser beam was removed immediately.

Laser Device Description

Thermal nociceptive thresholds were measured using a purpose-built remote laser device (M.P.B. Technologies Inc. Dorval, Canada). The carbon dioxide laser produced a 5mm diameter beam with a power output of 160mW and was aimed using a visible helium laser. The device was set to heat a thermocouple (Handheld K-type Thermocouple Indicator, TC Direct, Australia) to 63°C within 15 s. Temperature measurements were taken from a distance of 2m from the thermocouple at three, five, seven, nine, 11, 13 and 15 s to ensure that the temperature increased at a constant rate. The temperature of the laser was also tested at the same time point (8 s) over various distances (1.0 - 3.1 m at intervals of 30 cm), to ensure that the laser stimulus didn't attenuate over distance. No difference in temperature across distances was found.

Statistical Analysis

All 225 data points (3 tests per lamb) were included in the statistical analysis. To account for the Type 1 censoring of the data (maximum latency of 15s to respond), they were analyzed using an accelerated failure time model assuming a lognormal distribution (Collett, 2003). In the absence of censoring this would be equivalent to fitting a normal regression model to the log transformed latencies. To allow for variation between lambs, a frailty term (i.e. a random effect for Lamb) was included in the model (Collett, 2003), from which the effects of age, sex and their interaction were evaluated. The analysis was performed using R Statistical Software (R Development Core Team, 2011).

Results

Neither age ($Z = -1.08$, $P = 0.28$) nor sex ($Z = -0.64$, $P = 0.52$) had a significant effect on the lambs' median latency to respond to the thermal stimulus. However, there was a significant age*sex interaction ($Z = 2.35$, $P = 0.02$). The median latency to respond was similar for males and females in the days following birth. Thereafter, the response times of males and females diverged, with latency to respond increasing significantly with age in male lambs while female response latency decreased with age, but not significantly (Figure 6). The variance of the log scale time has two components. The variance within lambs is 0.853 lognormal and the variance between lambs is 0.508 lognormal.

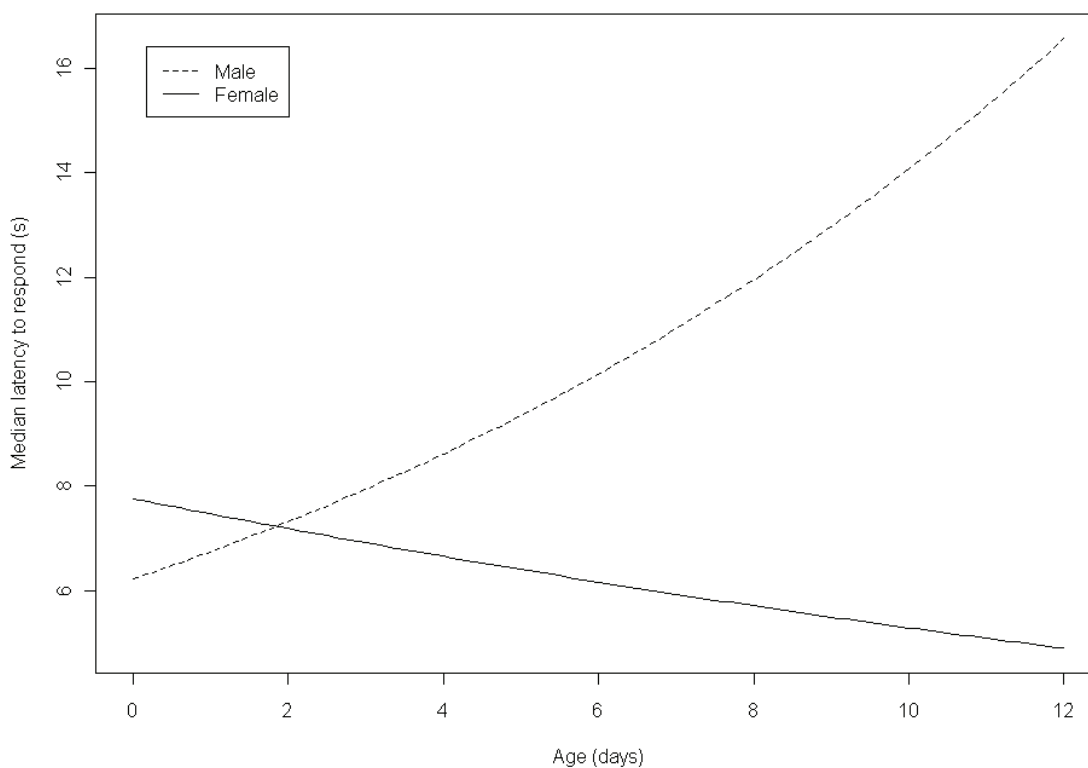


Figure 6: Median latency of males and females to respond (s) to thermal stimulation depending on age of testing (days).

Discussion

This study aimed to investigate effects of age and sex on baseline pain sensitivity in lambs using a thermal stimulus. We hypothesized that older lambs would be more sensitive to pain than younger lambs and that female lambs would be more sensitive to pain than male lambs. While there was no overall effect of sex or age, these factors had an interactive effect on pain sensitivity. Following birth, males become less sensitive to pain with increasing age, whereas females' pain sensitivity did not change significantly. At older ages, males appeared to be less sensitive to pain than females.

An interaction effect was also found in rats, where sex differences in pain sensitivity emerged only with increasing age (Beatty and Fessler, 1975). However, in contrast to our study, in which sensitivity of males and females diverged with age, both male and female pain sensitivity decreased with age in rats, with male sensitivity decreasing more rapidly than females' (Beatty and Fessler, 1975). The variance between studies may be explained by the use of different nociceptive modalities (electrical versus thermal) or may relate to differences in neural development in altricial and precocial species.

The direction of the sex difference in older animals was the same in rats and lambs. In both cases, older females appeared more sensitive to pain than older males. These findings are in agreement with other studies of adult mammals (Chesterton et al., 2003; Fillingim and Maxiner, 1995; Mogil et al., 2000; Negus et al., 2004; Riley et al., 1998). However, Cook (1997; 1998) found no sex differences in baseline thermal pain sensitivity in sheep at any age, although there was a sex difference in opioid analgesia. It would be interesting to extend our study by using older animals to see if the sex differences observed using our laser model persist in adult sheep.

This research is novel in that it suggests that differences in pain sensitivity between males and females are not innate in lambs, but rather develop over the first weeks of life. It is unclear precisely how hormones, physiology, genetics and environment interact and influence sex differences in pain sensitivity. We propose three interpretations for the ontogeny of sex differences in pain sensitivity in lambs: sex differences in the ontogeny of nociceptive processing; sex differences in the ontogeny of other physiological processes relevant to thermal nociception; or mediation of pain sensitivity by social factors which differ according to sex.

Sex differences in pain sensitivity may relate to the ontogeny of the pain processing system, which may vary between male and female lambs. Sex-specific changes may occur in peripheral components of pain processing systems, for example in the population, distribution or sensitivity of cutaneous nociceptive fibres, or centrally through the integration of nociceptive signals.

For example, human studies provide evidence that the central processing of pain differs between sexes, with greater integration of nociceptive signals by central neurons in females compared to males (Sarhani et al., 2004). Little is known about the ontogeny of nociceptive processing in vertebrates, so further investigation may be required to test this interpretation.

Alternatively, the development of sex differences in pain sensitivity with age could be explained by ontogeny of other physiological processes relevant to thermal nociception. For instance, variation between the sexes in the absorption of radiant heat from the laser could occur due to sex-specific changes in skin thickness or peripheral thermoregulation over the first two weeks of life. In humans, increasing skin thickness necessitated an increase in the power output of the CO² laser to elicit the same nociceptive threshold (Arendt-Nielsen and Bjerring, 1988). Therefore, a larger increase in skin thickness with age in male lambs could have influenced their latency to respond to the laser stimulus.

It may also be that male lambs develop effective peripheral thermoregulatory capabilities earlier in life than do female lambs. This would allow older males to reduce circulation to the limbs more effectively in cooler ambient temperatures (i.e. vasoconstriction), meaning that their initial skin temperature would be lower than that of females and younger males. In turn, more heat energy would need to be applied for the limb tissues to reach the temperature at which thermal nociceptors are activated. Hence, a longer application of the laser may have been required to elicit a behavioural response from older males relative to younger males, and this may account for the larger percentage of older males that did not respond in at least one test (80% of 12 day old males failed to respond in at least one test compared to 14% of females).

During our study, ambient temperature ranged from 3.1 to 17.3°C; however, there is no published information on sex differences in thermoregulatory abilities in lambs. Direct measurement of initial skin temperature, as well as conducting testing in a relatively constant ambient temperature would help resolve these issues.

Lastly, the divergence of pain sensitivity between males and females may be mediated by social factors. Males may have similar nociceptive thresholds to females but may not outwardly express pain behaviour in the same way that females do (Greenspan et al., 2007). Alternatively, maternal care during the lamb testing may have influenced pain sensitivity. Physical proximity to, and increased behavioural synchrony with, the ewe have both been associated with decreased sensitivity of lambs to a thermal stimulus (Hild et al., 2010). However, based on the observation that male lambs interact less frequently with the ewe in weeks two to four of life (Guilhem et al., 2005), we would expect them to be more sensitive than females at this age, which was not the case.

Interestingly, the ontogeny of sex differences in pain sensitivity reported in this study parallels the development of sex differences in activity and play behaviour in lambs (Sachs and Harris, 1978), suggesting that the two phenomena may be related. For example, males head-butt more often as juveniles and this frequency decreases with age, whereas females head-butt infrequently when they are young and then more often than males as adults. The authors suggest that gonadal or pituitary hormone differences between the sexes accounts for differences in play. Those hormones may also underlie the ontogeny of pain sensitivity, although this is yet to be investigated.

The high density of innervation in the area we tested (Kirk et al., 1987) means the likelihood of activating nociceptors with a 5mm laser beam was high. Therefore, it is unlikely that we 'missed' nociceptive nerves fibres in those lambs that failed to respond. Instead, the temperature of the skin surrounding the nociceptors was probably less than expected based on the thermocouple measurements. Carbon dioxide radiation is fully absorbed within the superficial skin layer (Arendt-Nielsen and Bjerring, 1988) and heat may be dissipated through the circulatory system. Hence the temperature of the skin overlying the lateral metatarsus may have been less than the expected 63°C at 15 seconds. This is likely as no tissue damage was observed on any of the lambs, and may account for the 32% of lambs overall that did not respond in at least one test. Again, direct measurement of skin temperature would help elucidate the mechanisms underlying the observed sex differences.

Conclusion

The results of this study suggest that differences in the pain sensitivity of male and female lambs are not, as expected, present from birth but rather may be the result of post-natal development. If confirmed, these findings suggest that the most appropriate age to perform painful husbandry procedures may vary according to sex. For example, it may be most appropriate to tail dock females at younger ages but castrate and dock males later. In addition, sex differences in pain sensitivity may necessitate different analgesic strategies for older males and females to minimize pain induced by husbandry procedures.

Future studies should use thermal imaging of skin to more accurately measure the effect that laser stimulation has on the skin temperature of lambs as well as conduct testing at a constant ambient temperature. The range of ages should be expanded to include older lambs and adult sheep to see if pain sensitivity continues to diverge and whether sex differences persist, thus providing a more complete picture of the ontogeny of pain processing development.

Social context and other factors influence the behavioural expression of pain by lambs

3.2

This chapter is based on the following paper:

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Abstract

There is evidence that the presence of a conspecific can alter the experience of pain in humans and other animals. This ‘social buffering’ may be mediated by factors such as relatedness and familiarity. This study investigates whether and how the social context affects the behavioural response of lambs to painful tail-docking. Specifically, we investigated whether the presence of a lamb that is familiar, related or neither, and previous experience of the test environment affected pain expression. Forty-four lambs were reared to allow testing in one of three social conditions: Familiar Related (FR, twins), Familiar Unrelated (FU) or Unfamiliar Unrelated (UU). Each lamb was exposed to the test environment twice over two rounds, once as the actor (i.e. tail-docked) and once as the observer (not tail-docked). The pain-related behaviour of the actor lamb, as well as where it was looking was recorded before and after tail-docking. As expected, all docked lambs showed an increase in the frequency of active behaviours previously associated with docking pain, an increase in the time spent in abnormal postures and decrease in time spent in normal postures. However, lambs tested with a familiar, related partner (twin) showed a smaller increase in rolling than the other groups (mean ranks of change \pm SE: FR 16.4 ± 3.1 , FU 26.3 ± 2.6 , UU 26.0 ± 3.2). In addition, lambs who had previously experienced the test environment showed overall less activity and a smaller increase in active behaviours after docking than those docked on their first exposure (e.g. round 1 and 2 mean ranks \pm SE: jump 49.4 ± 2.9 , 40.4 ± 3.0 ; looking at own tail 49.6 ± 2.5 , 39.7 ± 2.6 ; round 1 and 2 mean ranks of change \pm SE: headshake 28.2 ± 2.3 , 16.73 ± 2.5 ; abnormal upright 29.0 ± 2.5 , 15.8 ± 2.6). This is the first study to demonstrate that the occurrence of social buffering on lamb pain behaviour depends on the relationship between the actor and observer and on previous experience of the test environment.

Keywords: Lamb; Pain; Social Context; Social Buffering

Introduction

The behaviour of animals living in a social group may be influenced, not only by their physical environment, but also by the more immediate social context (Dall et al., 2005). Social context refers to the individuals who are immediately present at a particular time, such as parents or other conspecifics (Dall et al., 2005). More specifically, the presence of a conspecific (here-after termed “observer”) can alter the pain, fear or stress states of an individual (here-after termed “actor”). For example, the presence of an observer may have an analgesic or calming effect on the actor (Langford et al., 2010). Such “social buffering” has been demonstrated in a number of species (Kikusui et al., 2006) including humans (Thorsteinsson et al., 1998), non-human primates (Coe et al., 1982; Levine et al., 1997; Mendoza et al., 1978), guinea pigs (Hennessy et al., 2000), rats (Davitz and Mason, 1955; Kiyokawa et al., 2012; Morrison and Hill, 1967) and sheep (Ligout and Porter, 2004; Porter et al., 1995). Social buffering of pain states is of particular interest, as it may allow animals to recover more quickly from aversive experiences (Kikusui et al., 2006). In addition, social buffering in this context may be ultimately beneficial as it reduces the behavioural signs of pain in conspecifics, thus reducing the likelihood of possible predation (Hamilton, 1964).

In rodents, the effect of social buffering on pain behaviour has been shown to be mediated by familiarity or relatedness between the actor and observer as well as sex. Mice were less sensitive to thermal pain when tested with a familiar, related individual compared with an unfamiliar, unrelated test-mate (D’Amato, 1998). Mice also displayed fewer pain-related behaviours after being injected with formalin or acetic acid in the presence of a familiar individual than when alone or with an unfamiliar mouse (Gioiosa et al., 2009; Langford et al., 2006; Langford et al., 2010). The sex of the mice also mediated the social buffering effect. Mice displayed fewer pain-related behaviours if both actor and observer were male and unfamiliar with each other than if they were both female. The authors suggest this is due to the stress or distraction associated with the presence of another male (Langford et al., 2006).

Previous work describing social buffering for pain has focused on rodent models, but sheep also make a good study species to understand behaviour and the social lives of animals. Domestic sheep are a social species with a strong tendency to form groups (Esztevez et al., 2007), meaning they are often in close proximity to one another which may facilitate social buffering. Being a prey species, there may be adaptive value to being able to recover quickly from painful experiences, for example, by drawing less attention to the individual in pain, and subsequently to the flock, or being able to regain function in order to escape from a predator sooner. In addition, sheep routinely undergo painful husbandry procedures such as tail docking, ear tagging and castration.

There are mixed findings regarding the effects of social context on pain expression in sheep. While the presence of the ewe decreased the intensity of pain expression in lambs (Hild et al., 2010), the presence of a familiar, but unrelated, observer lamb had no analgesic effect on physiological and behavioural indicators of pain in castrated lambs (Colditz et al., 2012). This suggests that, like in rodents, the relationship between the observer and actor determines the effect of social context on pain expression. However, this has never been specifically tested in any species other than rodents.

This study investigated whether and how the social context affected the behavioural response of lambs to docking. Specifically, we evaluated the effects of observer relatedness and familiarity, the actor's previous experience of the test environment and procedure, and the sex of the actor and observer on the expression of pain behaviour. We hypothesized that lambs would show a reduced behavioural response to docking, indicative of social buffering, when tested with a familiar, related partner compared to an unrelated partner.

Methods

Animals and General Care

All procedures were approved by the Massey University Animal Ethics Committee (Protocol 10/24). The study was undertaken at the AgResearch Whatawhata farm in Hamilton, New Zealand in August 2010. Forty-five, mixed age, Romney cross ewes and 65 of their single and twin lambs were used in this study. Prior to lambing and in the 4 weeks prior to testing, the ewes and lambs were kept on pasture according to normal husbandry practice. Tail-docking and observations of behaviour were undertaken in covered yards with dirt floors.

After birth, ewes and lambs were left undisturbed for at least 3 h to facilitate bonding and suckling. Within the first 24 h after birth, ewe/lamb pairs were brought into a covered area and allocated to one of three treatment groups. Each lamb had a unique identification number sprayed on its back while ewes were identified by their ear tags. Date of birth, ewe tag number, treatment group, sex and whether the lamb was a single or twin were recorded. Ewes and lambs were then moved into one of three new paddocks according to their treatment group; the pairs were kept in these paddocks for 4 weeks before the first round of testing began. During these 4 weeks, 15 of the 65 lambs died or were excluded from the study because one twin in a related pair to be tested together died so the other could no longer be tested ($n = 9$) or because the sex of the lamb had not been recorded ($n = 6$).

2.2 Treatment

Fifty lambs were allocated to one of three treatment groups: Familiar Related (FR, n=8 pairs), Familiar Unrelated (FU, n=9 pairs) or Unfamiliar Unrelated (UU, n=8 pairs). These groups reflected the test lamb's relatedness to, and presumed familiarity with, the other lamb with which it was tested at tail-docking (test-mate). FR test-mates were twins (i.e. siblings born on the same day) and therefore were kept in the same paddock with their dam during the 4 weeks before the first round of testing (i.e. familiar). FU test-mates were kept in the same paddock for 4 weeks (familiar) but were from different ewes (unrelated). UU test-mates were kept in separate paddocks (unfamiliar) and were unrelated to each other. A large flock of these ewes were mated to multiple rams. Thus, there is a chance that some FU and UU lambs had the same sire and/or that their dams were related. However, these relationships would have been randomly distributed across the three treatment groups and thus would be unlikely to systematically influence the results.

Treatment paddocks were similar in size, approximately 80 m by 50 m. Two paddocks (A and C) were adjacent to one another, whereas the other (B) was separated from A and C by a dirt road. The FR and FU groups were kept in paddocks A and C, respectively. Lamb/ewe pairs in the UU group were kept half in paddock A and half in paddock B; UU lambs were tested with a lamb from the other paddock so that there had been no close social contact between them before testing.

We attempted to balance lamb sex over the three treatment groups, and to balance the numbers of singletons and twins in the FU and UU groups (Table 12). Ideally, we wanted all FU and UU lambs to be singletons to simplify the testing procedure, i.e. to avoid the other twin distracting the ewe during testing. However, because of the large number of twins born, it was necessary to allocate lambs with siblings to the FU and UU groups (Table 12). In these cases, twins were paired for testing with other twins from a different ewe. One lamb from each twin pair was randomly selected to be tested; the other twin from each pair was excluded from testing altogether.

Experimental Set Up

Each lamb was exposed to the test environment twice, once as the actor (i.e. tail-docked) and once as the observer (not tail-docked). Lambs were docked in one of two rounds, so that half the lambs were docked in their first exposure to the test environment (round 1, 4-5 weeks old) and half were docked in their second exposure (round 2, 6-7 weeks old). Round 1 started when lambs were approximately 4 weeks old and was completed over 8 consecutive days. Each day, three or four pairs were tested, with at least one pair from each treatment group to minimize any effects of day of testing on behaviour.

Round 2 started 6 days after the end of round 1, when lambs were 6 weeks old. Pairs were retested in the same order as round 1. This ensured that each lamb docked in round 1 had 14 days to recover from docking before it was exposed to the test environment again. In round 2, the lamb that had been the actor in round 1 now became the observer and vice versa.

Testing Procedure

On the day of testing, lambs and dams were brought into the covered area one paddock at a time. The lambs to be tested and their dams were separated from the rest of the flock and brought, one at a time, into the barn. The remaining animals were returned to the paddock. In the barn, the two lambs to be tested were separated from their dams and placed together in a 2 m² pen. Their dams were held together in an adjacent 4 m² pen and were provided with Fiber Pro lucerne and molasses feed (Fiber Fresh Feeds Ltd., Reporoa, New Zealand) and water. The sides of the pens were wooden bars so that the lambs and dams had visual, olfactory and limited physical access to each other throughout testing. When FU and UU twins were tested the remaining twin or twins were put in the adjacent pen with the dams.

Once inside the test pen, one of the lambs was randomly selected and a dot was painted on its shoulder to denote that it would be docked (actor). A video camera (Sony Handycam DCR-SR20, Sony Electronics Asia Pacific Pte Ltd. Tokyo, Japan) was set up at the front of the test pen and angled to capture as much of the pen as possible. The lambs were allowed 30 min to settle, undisturbed by human presence, before recording commenced.

After the settling time, undisturbed lamb behaviour was video-recorded for 30 min at 24 frames/s before tail-docking (pre-docking period). The behaviour of both the actor and observer lambs was recorded, however only the data from the actor lamb are presented here. One researcher then entered the pen and restrained the actor lamb to allow another researcher to apply the docking ring. The rubber ring was applied using an elastrator between two tail vertebrae at a point allowing sufficient tail proximal to the ring to cover the anus (and vulva for female lambs). When the ring had been applied, the researchers left the pen, and lamb behaviour was recorded for a further 30 min (post-docking period). Lambs and dams were then released from the pens and returned to their allocated paddock.

Analysis of Actor Lambs' Behaviour

Videos were edited to produce two, 30-min files per actor lamb (pre- and post-docking periods). For each 30-min file, event and state behaviours were scored continuously according to Table 13. Event behaviours were scored as the total number of times each behaviour occurred in 30 min. State behaviours were scored as the proportion of time spent engaging in each behaviour. Some actor lambs spent a small proportion of time out of view of the camera (85% of lambs were in view for 90-100% of the 60 min of video, 8% were out of view 10-38% of the time).

Six actor lambs were out of view for more than 38% of the video recording time and their data were excluded from the analysis (final $n=44$, Table 12). For the remaining lambs the proportion of the total in-view time the lamb spent displaying each state behaviour was calculated and analyzed.

Videos were scored in a random order and the video observer was semi-blind to treatment group and recording period; the camera also partially captured the dams and any siblings in the background (two dams would have denoted either a FU or UU treatment group as opposed to a FR group) and because of the behaviour of the lamb in the post-docking period, the video observer may have been able to deduce the treatment group and period.

Table 12: The number of male, female, singleton and twin lambs in each treatment group (FR= Familiar/related, FU= Familiar/unrelated, UU= Unfamiliar/unrelated) included in the analysis.

Treatment group	Sex	Singletons	Twins	TOTAL
FR	M	0	6	6
	F	0	7	7
FU	M	6	3	9
	F	7	1	8
UU	M	6	3	9
	F	3	2	5
TOTAL		22	22	44

Statistical Analysis

All statistical analyses were performed using SAS Version 9.2 (SAS Institute Inc., North Carolina, USA). Pre/post (period) data could not be transformed to satisfy assumptions for parametric analyses, therefore repeated measure analyses were performed on ranked data. A MIXED model was used to evaluate the effects of tail docking on pain-related behaviour, with period as the repeated measure, lamb as the random effect, and treatment (FR, FU, UU), round (1, 2) and pair-sex (SameF= both lambs female; MixedAcM= actor lamb is male, observer is female; SameM= both lambs male, MixedAcF= actor lamb is female, observer is male) as fixed effects.

As well as analyzing ranked data from the pre- and post-docking periods, the change in frequency or duration of each behaviour after docking (post – pre) was calculated and analyzed. Because we were interested in the effects of independent variables on the relative magnitude of change due to docking, change variables were calculated and then ranked. Ranked change data were analyzed using factorial ANOVA with treatment, round and pair-sex as independent variables. When significant effects were found, post hoc tests were performed using Bonferroni adjustment for multiple comparisons. Differences were considered significant at $P < 0.05$.

Table 13: Behaviours scored for actor (tail-docked) lambs. Pain-related behaviours based on Molony and Kent (1997).

Behaviour	Description
Visual Behaviours	
Looking at Ewe	Head and eyes turned in the direction of the ewe
Looking at Lamb	Head and eyes turned in the direction of the other lamb
Looking at Own Tail	Head and eyes turned in the direction of its own tail
Pain-related Behaviours	
Normal Upright	Standing, walking or playing while exhibiting a usual posture or gait; smooth movements
Abnormal Upright	Standing exhibiting unusual posture e.g. rounded, hunched appearance; ataxia; jerky movements; swaying; walking unsteadily; walking backwards; walking on knees
Normal Lying	Ventral recumbency, all legs tucked under body or very close to body
Abnormal Lying	Twisted lying: ventral recumbency with forelimbs tucked under body, one or both hind limbs partially or fully extended; including dog sitting
Lateral Lying	Lateral recumbency with one shoulder on ground, hind limbs and/or forelimbs fully extended
Repetitive Standing	Number of times lamb moved between lying and standing or part way thereof. One event comprised moving from: lying to kneeling or vice versa; kneeling to standing or vice versa; lying to standing or vice versa; dog sitting to standing or vice versa
Kick	While lying or standing, rapid extension of one or both hind limbs either away from or towards the body
Roll	Moving from lateral recumbency on one flank to the other. Rolls from flank to dorsum and back to same flank also counted as one event
Jump	All four feet off ground simultaneously, including jumping off hind limbs with forelimbs on pen walls
Stamp	Rapid forceful downward movement of fore or hind foot
Rump Wag	Wag of rump to shift weight, with or without wag of tail
Lip curl/Neck arch	While lying, curling upper lip (like flehmen) with or without arching of head back over the neck
Head Shake	Forceful voluntary shake of head
Headbutt	Forcefully shoving head into another lamb or object or performing action without contact

Results

Effects on repeated measures data

Tail docking resulted in a significant increase in the frequency of repetitive standing (Pre- and Post-docking mean ranks \pm SE: 26.2 ± 2.2 , 64.5 ± 2.2), jumping (37.5 ± 3.0 , 52.3 ± 3.0), stamping (31.7 ± 2.8 , 57.3 ± 2.8) and headbutt (34.9 ± 3.4 , 55.2 ± 3.4) regardless of treatment group, round or pair-sex. In addition lambs spent more time looking at their own tail (24.3 ± 2.3 , 65.0 ± 2.3), lying abnormally (24.4 ± 1.9 , 66.2 ± 1.9) and lateral lying (30.9 ± 2.5 , 59.0 ± 2.5) and less time in the normal upright posture (60.5 ± 3.4 , 28.1 ± 3.4) (Table 14).

Period and round had an interactive effect on the frequency of kicking, lip curling/neck arching and headshaking as well as on the proportion of time spent in the abnormal upright and normal lying postures (Table 14). In both rounds, kicking, lip curling/neck arching and time spent in the abnormal upright posture increased after docking. However, lambs kicked more often and spent more time in the abnormal upright posture in the post-docking period in round 1 than round 2 (Figure 7a and 7d). Only in round 1 did lambs headshake more and spend less time lying normally after docking (Figure 7c and 7e).

Table 14: Results of MIXED model on frequency or duration of actors' (tail-docked lambs') behaviour before and after tail docking (period). Factors included in the model are pair-sex (same-sex female, same-sex male, mixed-sex actor female, mixed-sex actor male), round (1, 2) and treatment (Familiar Related, Familiar Unrelated, Unfamiliar Unrelated). Bold text denotes a statistically significant result at $P < 0.05$.

Behaviour	Period		Pair-sex		Round		Treatment		Interactive Effects		
	F _(1,29)	P	F _(3,26)	P	F _(1,26)	P	F _(2,26)	P	Type _(df)	F	P
Visual Behaviours											
Looking at Ewe	0.99	0.33	0.61	0.62	3.39	0.08	0.56	0.58			
Looking at Lamb	2.39	0.13	2.44	0.09	1.19	0.29	0.71	0.50	Round*Pair-sex _(3,26)	4.78	0.009
Looking at Own Tail	169.08	<0.0001	0.34	0.80	7.04	0.01	0.15	0.86			
Pain-related Behaviours											
Normal Upright	68.66	<0.0001	0.08	0.97	0.70	0.41	0.04	0.96			
Normal Lying	6.06	0.020	0.29	0.83	0.24	0.63	2.04	0.15	Period*Round _(1,26)	11.21	0.002
Abnormal Upright	225.06	<0.0001	0.11	0.96	8.76	0.01	0.39	0.68	Period*Round _(1,26)	9.38	0.005
Abnormal Lying	256.86	<0.0001	0.67	0.58	2.07	0.16	0.14	0.87			
Lateral Lying	65.45	<0.0001	1.56	0.22	3.62	0.07	0.93	0.41			
Repetitive Standing	164.07	<0.0001	1.56	0.22	3.48	0.07	0.41	0.67			
Kick	210.12	<0.0001	0.23	0.88	8.65	0.01	0.76	0.48	Period*Round _(1,26)	5.91	0.02
									Treatment*Pair-sex _(3,26)	2.82	0.03
Roll	55.06	<0.0001	0.19	0.90	0.06	0.81	2.60	0.09	Period*Treatment _(1,26)	3.41	0.050
Jump	11.00	0.003	0.38	0.77	4.23	0.05	0.13	0.88			
Stamp	39.97	<0.0001	0.99	0.41	0.68	0.42	0.50	0.61			
Rump Wag	60.18	<0.0001	1.16	0.34	2.12	0.16	1.30	0.29	Period*Pair-sex _(1,26)	3.10	0.04
Lip curl/Neck arch	100.81	<0.0001	0.13	0.94	1.48	0.23	0.16	0.85	Period*Round _(1,26)	4.88	0.035
Headshake	24.36	<0.0001	1.21	0.33	7.48	0.01	1.08	0.35	Period*Round _(1,26)	4.70	0.04
Headbutt	26.83	<0.0001	1.50	0.24	0.59	0.45	3.74	0.04			

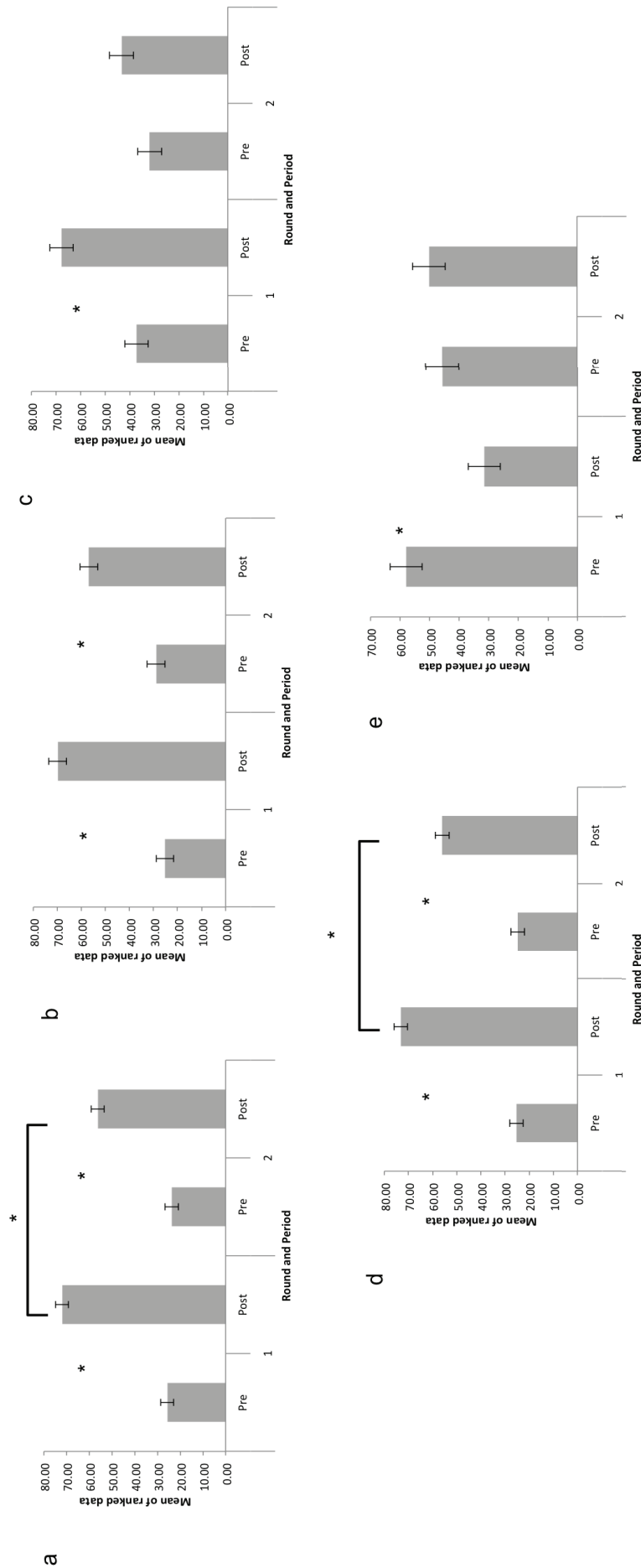


Figure 7: Mean ranks \pm SE frequency of kick (a), lip curl/neck arch (b), headshake (c) and the proportion of time in the abnormal upright (d) and normal lying (e) postures. A MIXED model was used to evaluate the effects of tail docking on pain-related behaviour, with period as the repeated measure, lamb as the random effect, and treatment (FR, FU, UU), round (1, 2) and pair-sex (SameF= both lambs female; MixedAcM= actor lamb is male, observer is female; SameM= both lambs male, MixedAcF= actor lamb is female, observer is male) as fixed effects. Significant differences at $P < 0.05$ are denoted by an asterisk (*).

Period and pair-sex had an interactive effect on the frequency of rump wagging (Table 14). All actor lambs, except female actor lambs in a mixed-sex pair (MixedAcF), wagged their rumps more frequently after docking. Female lambs in a mixed-sex pair also wagged their rumps significantly less after docking than females in a same-sex pair (Figure 8).

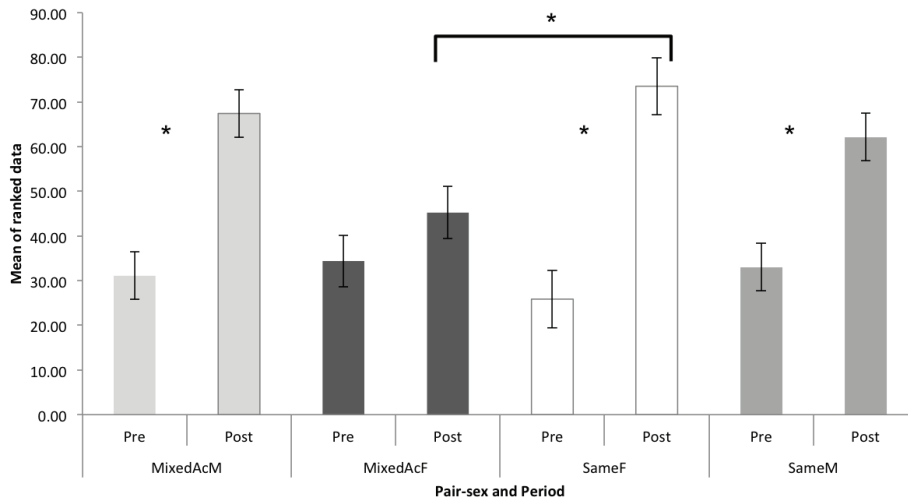


Figure 8: Mean ranks ± SE (period*pair-sex interactive effect) frequency of rump wag. MixedAcM= actor lamb is male, observer is female; SameM= both lambs male, MixedAcF= actor lamb is female, observer is male. Significant differences at $P < 0.05$ are denoted by an asterisk (*).

Period and treatment had an interactive effect on the frequency of rolling (Table 14). Both FU and UU lambs showed an increase in rolling after docking and rolled significantly more after docking than FR lambs (Figure 9).

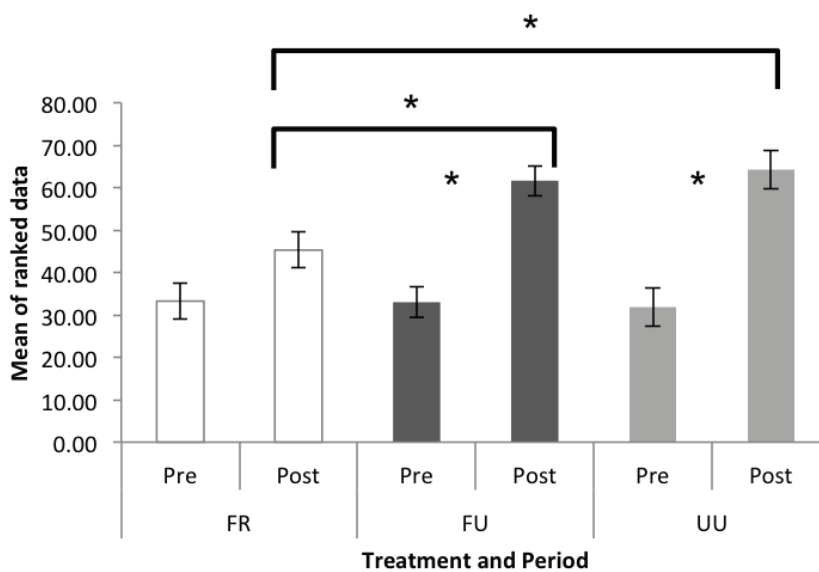


Figure 9: Mean ranks ± SE (period*treatment interactive effect) frequency of roll. FR = Familiar Related, FU = Familiar Unrelated, UU = Unfamiliar Unrelated. Significant differences at $P < 0.05$ are denoted by an asterisk (*).

Treatment affected the frequency of headbutting, with FR lambs headbutting less (Mean ranks \pm SE: 34.2 ± 5.0) over both periods than UU lambs (53.4 ± 5.5 , $t = -2.58$, $P = 0.05$).

Treatment and pair-sex had an interactive effect on the frequency of kicking. FR male lambs in a mixed-sex pair kicked less (Post-docking mean ranks for MixedAcM \pm SE: 36.2 ± 3.8) after docking than those in the FU treatment group (55.7 ± 3.8 , $t = -3.61$, $P = 0.04$).

Lambs jumped significantly more often (Round 1 and 2 mean ranks \pm SE: 49.4 ± 2.9 , 40.4 ± 3.0) and spent more time looking at their own tail (49.6 ± 2.5 , 39.7 ± 2.6) over both periods in round 1 compared to round 2 (Table 14).

Round and pair-sex had an interactive effect on the time spent looking at the other (observer) lamb. In round one, female lambs looked at a male observer more often than at a female observer (Round 1 mean ranks \pm SE: MixedAcF 63.8 ± 9.9 , SameF 21.6 ± 8.7 , $t = 3.24$, $P = 0.04$). Female lambs looked at a female observer less often in round 1 than in round 2 (SameF mean ranks \pm SE: round 1 63.6 ± 9.7 , round 2 63.6 ± 9.7 , $t = -3.23$, $P = 0.04$).

Effects on change data

Treatment affected the change in frequency of rolling ($F_{(2,43)} = 4.15$, $P = 0.03$). FR lambs tended to show a smaller increase in rolling after docking (Mean ranks of change \pm SE: FR 16.4 ± 3.1) than FU lambs (26.3 ± 2.6 , $t = 2.45$, $P = 0.06$).

Lambs in round 1 showed a greater increase in the frequency of headshaking (Round 1 and 2 mean ranks of change \pm SE: 28.2 ± 2.6 , 16.7 ± 2.5 , $F_{(1,43)} = 5.30$, $P = 0.03$) as well as in the time spent in the abnormal upright posture (29.0 ± 2.5 , 15.8 ± 2.6 , $F_{(1,43)} = 6.44$, $P = 0.02$) than lambs docked in round 2. Lambs docked in round 1 also showed a larger decrease in the time spent lying normally after docking than lambs docked in round 2 (26.0 ± 2.8 , 18.1 ± 2.7 , $F_{(1,43)} = 5.34$, $P = 0.03$).

Discussion

This study investigated whether and how the social context of the test environment and previous experience of the test environment affect the pain behaviour of lambs. We hypothesized that lambs would show lower levels of pain-related behaviour in response to docking, indicative of social buffering, when with a familiar, related partner as opposed to a familiar or unfamiliar unrelated partner.

We found a significant effect of period on all behaviours recorded, except looking at the other lamb and looking at the ewe. All docked lambs showed an increase in the frequency of active behaviours previously associated with docking pain, as well as an increase in the time spent in abnormal postures and a decrease in the time spent in normal postures. This is consistent with literature on behavioural responses to tail-docking and confirms that the behaviours we measured do reflect pain in lambs (Grant, 2004; Lester et al., 1996; Mellor and Stafford, 2000; Reefmann et al., 2009; Thornton and Waterman-Pearson, 1999, 2002).

Social context

In accordance with our hypothesis, we saw minor differences in the behavioural response to docking according to the relationship between the actor and observer. Lambs tested with their twin showed a smaller increase in kicking and rolling than lambs tested with a familiar but unrelated test-mate or an unfamiliar unrelated test-mate. Social buffering, where the presence of a conspecific has an analgesic effect on the actor, is a plausible proximate mechanism to explain this finding.

The interpretation that social buffering of pain behaviour in lambs depends specifically on the relatedness between the actor and observer is supported by another study. Colditz et al. (2012) found no evidence of social buffering in unrelated lambs undergoing different methods of castration considered to vary in the degree of pain caused. However, all lambs in that study were tested in the same pen as their dams. Because the presence of the dam influences pain behaviour in lambs (Hild et al., 2010), this social effect would likely predominate over any potential effect of lamb social buffering.

Social buffering between siblings may be promoted through tactile contact. Such contact has been demonstrated to result in opioid release, which produces analgesia (Kikusui et al., 2006). For example, in mice, physical contact between siblings reduced pain sensitivity (D'Amato and Pavone, 1993). These effects were not found however, for non-sibling mice who spent less time resting together and grooming each other than siblings (D'Amato and Pavone, 1996). A similar effect may be occurring in lambs, whereby greater pro-social tactile contact between twin lambs results in a degree of analgesia.

Ultimately, it may be beneficial for twin lambs to buffer one another's pain resulting in a reduction in behavioural signs of pain. While displaying certain pain-related behaviours may benefit the actor by eliciting care behaviour from some conspecific observers (Williams, 2002) such behaviours also risk alerting potential predators to an animal's vulnerable condition (Williams, 2002). Related individuals are more likely to engage in behaviours that promote amelioration of pain in the actor because they gain an indirect reproductive advantage by doing so (Hamilton, 1964). Pro-social behaviours expressed by an observer to reduce its sibling's expression of pain behaviour would minimize unwanted attention towards both, thus increasing the likelihood of survival for both itself and a closely related individual. In contrast, the risk of interacting closely with an unrelated conspecific in pain would likely outweigh any benefits. In sheep, such social buffering mechanisms may have been promoted through domestication, which selects for socio-positive behaviours (Hennessy et al., 2009).

In this study, head-butting was strongly influenced by the social environment, particularly the relatedness of lambs being tested. We found that over both periods, lambs tested with a twin head-butted less than did unrelated or unfamiliar lambs. Head-butting is used as an aggressive or defensive behaviour by sheep (Lynch et al., 1992), which may be why unfamiliar lambs display this behaviour more. It is likely that head-butting in this case reflects aggression due to unfamiliarity as opposed to pain. As head-butting is often used as a behavioural indicator of pain (Molony and Kent, 1997), this result indicates the need to take social context into account when inferring pain using head-butting behaviour.

Round effects

As well as social context, round affected the expression of pain in the test environment. Lambs docked on their second exposure to the test environment displayed a smaller increase in active pain behaviours such as kicks, head shakes and lip curls after docking and spent less time standing abnormally and more time lying normally than did lambs docked on their first exposure. In addition, lambs in round 2 were less active over both periods and they jumped and looked at their own tail less often than did lambs in round 1.

The lower overall activity and less pronounced response to docking in round 2 may be due to either the greater familiarity with the test environment. When the environment is novel lambs are more active (Miranda-de la Lama et al., 2012) and may also spend more time actively trying to get to their dams in order to minimize the distress caused by separation. Lambs in the novel environment also showed some indication of greater pain perception (they lip curled more often and spent more time standing abnormally). Likewise there is some evidence in rats that non-harmful stress, such as being in a novel environment, increases pain sensitivity and the frequency of pain-related behaviour (Vidal and Jacob, 1982).

The other possibility is that the interaction between the two lambs had changed due to previous experience. In round 2, the now-observer had experienced pain in the test environment before and subsequently may act differently towards its test partner. The actor in round 2 may also act differently in the test environment and when docked, as it had observed pain behaviour there before.

While age may be a possible explanation for the lower overall activity in round 2, it is unlikely that the difference in pain-related behaviour between round 1 and 2 was due to age. Older lambs (6 weeks) show more pronounced behavioural and physiological responses to injury than do younger lambs (0-3 weeks) (Johnson et al., 2009; Molony et al., 1993; Thornton and Waterman-Pearson, 2002). We would have therefore expected lambs docked in round 2 (6-8 weeks old) to be more expressive of pain, however our results do not suggest this is the case.

Sex effects

The sex of the actor and observer had some minor effects on behaviour. For example, observer sex affect rump wagging, a pain behaviour, after docking, with female lambs showing less wagging in the presence of a male observer than with a female observer. Similarly, Langford et al. (2006) found that male mice displayed fewer pain-related behaviours when the test-mate was male, due to stress or distraction. Female actor lambs also spent more time looking at a male observer over both periods in round 1. However, as there were no consistent effects on multiple behaviours, the relevance of these sex relationships for the expression of behaviour is currently unclear.

In order to investigate the conclusions of this study further, it might be useful to do a cross-fostering experiment. In the current study there was the chance that some FU and UU lambs had the same sire and/or that their dams were related. While the degree of relatedness among such 'cousins' would be smaller than between twins, twin lambs will also spend more time together than unrelated lambs in the same paddock, so it is unclear whether it is the genetic component of being a twin or the greater familiarity that facilitates social buffering in twin lambs. A cross-fostering experiment, in which twin lambs are reared apart or unrelated lambs are reared together, would help tease apart these effects. In addition, during testing, a control treatment could be included in which the lamb experiences pain by itself in either the first or second round and is just present in the environment in the other round. However, the effect of social isolation would need to be considered when interpreting the results of this treatment.

Conclusion

This is the first study to demonstrate that the occurrence of social buffering on lamb pain behaviour depends on the relationship between the actor and observer. In addition, there is some evidence that previous experience of the test environment affects the expression of pain as well as overall behaviour in a test environment. The finding that the presence of a twin reduces pain expression in an actor lamb may, ultimately, be explained by the benefit the lambs gain from minimizing unwanted attention towards them. Understanding the effects of social context and previous experience on pain behaviour also helps us to better interpret the results of other studies on pain behaviour.

How pain expression affects conspecific observer lambs



The previous chapter has focussed on one side of the social dyad, the actor lamb. This section looks at the other side, or how viewing an actor in pain affects the behaviour of an observer lamb. It also introduces a novel application of the Qualitative Behavioural Assessment technique for understanding the social behaviour in lambs.

Behavioural and emotional effects of viewing a conspecific in pain

4

This chapter is based on the following paper:

Guesgen, M.J., Beausoleil, N.J., Minot, E.O., Stewart, M., F. Wemelsfelder, Stafford, K.J. Behavioural and emotional effects of viewing a conspecific in pain. Behavioural Processes (in preparation).

Abstract

A change in behaviour by an observer, which is viewing and potentially responding to another animal, shown in the presence of an actor which is displaying a particular repertoire of behaviours, is referred to as socially facilitated behaviour (SFB). Such a change in observer behaviour may be brought about through the experience of empathy, the shared emotional state between two individuals which leads to the generation of an appropriate action. One stimulus of particular interest that can induce empathy is pain. The aims of this study were 1. to use both quantitative and qualitative behavioural assessment techniques to investigate whether lambs display socially facilitated behaviour (SFB) indicative of empathy when in the presence of a conspecific in pain and 2. explore how factors of familiarity, relatedness, sex and previous experience of pain may affect the expression of SFB. Fifty lambs were reared to allow testing in one of three social conditions: Familiar Related (FR), Familiar Unrelated (FU) or Unfamiliar Unrelated (UU). Each lamb was exposed to the test environment twice over two rounds, once as the actor (i.e. tail-docked) and once as the observer (not tail-docked). The behaviour of the observer lamb, as well as where the lamb was looking was recorded before and after tail-docking of the actor. In addition Qualitative Behavioural Assessment was used to describe changes in emotional expression of observer lambs. Both quantitative and qualitative measures provided some evidence that observer lambs display SFB when in the presence of a conspecific in pain and that doing so elicited a negative emotional state in observers. After actor docking, observers showed similar changes in some of the behaviours displayed by actors, in particular ear changes (Mean ranks \pm SE: Ears backward: pre 29.3 ± 3.2 , post 58.1 ± 3.2) and looking at their tail (Period*round effect: Round one: Round one: pre 28.7 ± 4.7 , post 61.1 ± 4.7 ; Round two: pre 40.7 ± 5.0 , post 43.3 ± 5.0). In addition, familiar related lambs looked at their tail more after actor docking than the other groups (FR: pre 36.4 ± 6.2 , post 64.2 ± 6.2 , FU pre 41.8 ± 5.2 , post 50.4 ± 5.2 , UU pre 45.9 ± 6.1 , post 44.1 ± 6.1) which is consistent with predictions of SFB. This is the first study to demonstrate SFB of lambs, possibly indicative of empathy, in response to conspecific pain.

Keywords: Empathy; Lamb; Pain; Socially Facilitated Behaviour; Social

Introduction

Animals that alter their behaviour based on observations of their environment are likely to enhance their survival or reproductive success (Stearns and Hoekstra, 2005). This is particularly so in a social environment, where the behaviour of a conspecific may act as a valuable signal or cue to an observer (Maynard Smith and Harper, 2003). In the simplest terms, animal interactions occur in a dyad, where one animal is the actor, displaying a particular repertoire of behaviours, and the other is the observer, viewing and potentially responding to the actor's behaviour.

A change in the behaviour of an observer in the presence of an actor is referred to as socially facilitated behaviour (SFB) (Clayton, 1978). Common examples of SFB in animals include changes in: food consumption (Dindo et al., 2009; Drewett, 2007; Zion et al., 2007); locomotion (Vogel et al., 1950); vigilance (Pays et al., 2009); and cleaning behaviours (Olsson et al., 2002). SFB is advantageous in a social environment as it acts to synchronize the activity of the group and maintain group cohesion (Clayton, 1978).

The expression of SFB may be achieved through empathy, the shared emotional state between two individuals, which leads to the generation of an appropriate action (Preston and de Waal, 2002). Empathy as defined here covers terms such as 'emotional contagion', 'emotional resonance' and 'affect matching' (Panksepp and Panksepp, 2013). Underlying this level of empathy are the neural networks that are activated both when an individual experiences an emotion, and when it views that emotion in a conspecific (Baird et al., 2011; Casile et al., 2011; Decety, 2010; Decety and Meyer, 2008; Ferrari et al., 2005). Activation of such systems will then lead to the generation of an appropriate response i.e. socially facilitated behaviour. This may be expressed in the form of "mirroring" behaviour, where the observer displays the same behaviour as the actor.

One stimulus of particular interest that may elicit empathy or SFB is pain behaviour. There is evidence that mice display SFB associated with pain. For example, observer mice showed writhing (a pain behaviour) when viewing another mouse writhing after injection of an irritating substance into the abdomen, although they themselves had not been injected (Langford et al., 2006). In addition, the magnitude of writhing behaviour of both the actor and observer was greater when both mice were injected simultaneously than when only one mouse of the pair had been injected or when the actor was injected in isolation. This suggests writhing behaviour was contagious, or in other words that empathic processing was occurring. In addition, it highlights that the interaction between the actor and observer goes both ways as the presence of the observer also altered the behaviour of the actor.

The expression of SFB may depend on features of the relationship between the actor and observer such as their relatedness, familiarity and sex, as well as previous experience of pain. Related individuals are more likely to interact with one another, as well as exhibit altruistic, or helping, behaviour as this may confer a fitness benefit (Hamilton, 1964).

In contrast, mice who were unfamiliar did not approach their cagemate experiencing pain more than they approached an unaffected conspecific (Langford et al., 2010). The value of expressing a particular behaviour may vary according to the sex of the social partner (Halliday, 1981). For example, mice writhed less if both mice were male and unfamiliar with each other than if they were familiar or both female. The authors suggest this is due to the stress or distraction associated with the presence of another male (Langford et al., 2006). Mice have also been shown to exhibit pro-social behaviour, in the form of closer proximity to a pained mouse, or towards a trapped cagemate in pain but only when the observer was female (Watanabe, 2012). This behaviour was only significant for females in one of these studies, with males spending equal time with a free cagemate as a trapped one (Langford et al., 2010). Previous experience can also modulate the expression of empathy. For example only rats that had previously experienced pain themselves displayed freezing behaviour and ultra-sonic vocalizations, indicative of empathy, when viewing a partner in pain (Atsak et al., 2011).

Previous work on non-human empathy associated with pain has focused on rodent models, but there is reason to believe that sheep may also experience empathy in such situations. Domestic sheep are a social species with a strong tendency to form groups (Esztevez et al., 2007). Although they are domestic, they still maintain strong anti-predator behaviours (Hansen et al., 2001; Penning et al., 1993). There are mixed findings regarding the expression of SFB by observer sheep. While ewes responded differently to lambs in pain than to lambs being handled (Edgar et al., 2010), the presence of an unrelated lamb in pain had no effect on the behaviour or physiology of an observer lamb who was also experiencing pain (Colditz et al., 2012). This suggests that, like in rodents, the relationship between the observer and actor determines the effect of an actor in pain on an observer sheep.

There are no prescribed descriptors for empathic behaviour in lambs. Therefore a qualitative assessment technique may be useful alongside a quantitative ethogram method for evaluating sheep empathy associated with pain. Qualitative Behavioural Assessment (QBA) is a 'whole animal' approach which integrates many features of the animal's behaviour such as body posture and movement as well as context to assess their response to an event or situation (Wemelsfelder et al., 2001). Numerous studies utilizing this technique have provided evidence that it is a valid and reliable method for exploring emotional states in a range of animals including sheep (Cockram et al., 2012; Napolitano et al., 2012; Phythian et al., 2013; Rutherford et al., 2012; Stockman et al., 2011; Stockman et al., 2012; Wemelsfelder et al., 2012; Wickham et al., 2012). QBA may therefore provide insight into the potential effect viewing a conspecific in pain has on the behavioural expression and emotional experience of lambs.

The aims of this study were to use both a quantitative technique and QBA to investigate whether lambs display socially facilitated behaviour (SFB) indicative of empathy when in the presence of a conspecific in pain and how factors of familiarity, relatedness, sex and previous experience of pain may affect the expression of SFB in this context.

Methods

Animals and General Care

All procedures were approved by the Massey University Animal Ethics Committee (Protocol 10/24). The study was undertaken at the AgResearch Whatawhata farm in Hamilton, New Zealand in August 2010. Forty-five, mixed age, Romney cross ewes and 50 of their single and twin lambs were used in this study. Prior to lambing and in the 4 weeks prior to testing, the ewes and lambs were kept on pasture according to normal husbandry practice. Tail-docking and observations of behaviour were undertaken in covered yards with dirt floors.

After birth, ewes and lambs were left undisturbed for at least 3 h to facilitate bonding and suckling. Within the first 24 h after birth, ewe/lamb pairs were brought into a covered area and allocated to one of three treatment groups. Each lamb had a unique identification number sprayed on its back while ewes were identified by their ear tags. Date of birth, ewe tag number, treatment group, sex and whether the lamb was a single or twin were recorded. Ewes and lambs were then moved into one of three new paddocks according to their treatment group; the pairs were kept in these paddocks for 4 weeks before the first round of testing began.

Treatment

Fifty lambs were allocated to one of three treatment groups: Familiar Related (FR, n=8 pairs), Familiar Unrelated (FU, n=9 pairs) or Unfamiliar Unrelated (UU, n=8 pairs). These groups reflected the test lamb's relatedness to, and presumed familiarity with, the other lamb with which it was tested at tail-docking (test-mate). FR test-mates were twins (i.e. siblings born on the same day) and therefore were kept in the same paddock with their dam during the 4 weeks before the first round of testing (i.e. familiar). FU test-mates were kept in the same paddock for 4 weeks (familiar) but were from different ewes (unrelated). UU test-mates were kept in separate paddocks (unfamiliar) and were unrelated to each other. A large flock of these ewes were mated to multiple rams. Thus, there is a chance that some FU and UU lambs had the same sire and/or that their dams were related. However, these relationships would have been randomly distributed across the three treatment groups and thus would be unlikely to systematically influence the results.

Treatment paddocks were similar in size, approximately 80 m by 50 m. Two paddocks (A and C) were adjacent to one another, whereas the other (B) was separated from A and C by a dirt road. The FR and FU groups were kept in paddocks A and C, respectively. Lamb/ewe pairs in the UU group were kept half in paddock A and half in paddock B; UU lambs were tested with a lamb from the other paddock so that there had been no close social contact between them before testing.

Ideally, we wanted all FU and UU lambs to be singletons to simplify the testing procedure, i.e. to avoid the other twin distracting the ewe during testing. However, because of the large number of twins born, it was necessary to allocate lambs with siblings to the FU and UU groups. In these cases, twins were paired for testing with other twins from a different ewe. One lamb from each twin pair was randomly selected to be tested; the other twin from each pair was excluded from testing altogether.

Experimental Set Up

Each lamb was exposed to the test environment twice, once as the actor (i.e. tail-docked) and once as the observer (not tail-docked). Lambs were docked in one of two rounds, so that half the lambs were docked in their first exposure to the test environment (round 1, 4-5 weeks old) and half were docked in their second exposure (round 2, 6-7 weeks old). Round 1 started when lambs were approximately 4 weeks old and was completed over 8 consecutive days. Each day, three or four pairs were tested, with at least one pair from each treatment group to minimize any effects of day of testing on behaviour.

Round 2 started 6 days after the end of round 1, when lambs were 6 weeks old. Pairs were retested in the same order as round 1. This ensured that each lamb docked in round 1 had 14 days to recover from docking before it was exposed to the test environment again. In round 2, the lamb that had been the actor in round 1 now became the observer and vice versa.

Testing Procedure

On the day of testing, lambs and dams were brought into the covered area one paddock at a time. The lambs to be tested and their dams were separated from the rest of the flock and brought, one at a time, into the barn. The remaining animals were returned to the paddock. In the barn, the two lambs to be tested were separated from their dams and placed together in a 2 m² pen. Their dams were held together in an adjacent 4 m² pen and were provided with Fiber Pro lucerne and molasses feed (Fiber Fresh Feeds Ltd., Reporoa, New Zealand) and water. The sides of the pens were wooden bars so that the lambs and dams had visual, olfactory and limited physical access to each other throughout testing. When FU and UU twins were tested the remaining twin or twins were put in the adjacent pen with the dams.

Once inside the test pen, one of the lambs was randomly selected and a dot was painted on its shoulder to denote that it would be docked (actor). A video camera (Sony Handycam DCR-SR20, Sony Electronics Asia Pacific Pte Ltd. Tokyo, Japan) was set up at the front of the test pen and angled to capture as much of the pen as possible. The lambs were allowed 30 min to settle, undisturbed by human presence, before recording commenced.

After the settling time, undisturbed lamb behaviour was video-recorded for 30 min at 24 frames/s before tail-docking (pre-docking period). The behaviour of both the actor and observer lambs was recorded, however only the data from the observer lamb is presented here. One researcher then entered the pen and restrained the actor lamb to allow another experimenter to apply the docking ring. The rubber ring was applied using an elastrator between two tail vertebrae at a point allowing sufficient tail proximal to the ring to cover the anus (and vulva for female lambs). When the ring had been applied, the researcher left the pen, and lamb behaviour was recorded for a further 30 min (post-docking period). Lambs and dams were then released from the pens and returned to their allocated paddock.

Quantitative Analysis of Observer Lambs' Behaviour

Videos were edited to produce two 30 min files per observer lamb (pre- and post-docking periods). For each 30 min file, behaviours that would indicate pain in a docked lamb (pain-related behaviours) and visual behaviours were scored continuously according to Table 15. Event behaviours were scored as the total number of times each behaviour occurred in 30 min. State behaviours were scored as the proportion of time spent engaging in each behaviour. Some observer lambs spent a small proportion of time out of view of the camera (86% of lambs were in view for 90-100% of the 60 min of video). Three observer lambs were out of view for more than 35% of the 60 min video recording time and their data were excluded from the analysis (final $n=47$, Table 16a). For the remaining lambs the proportion of the total in-view time the lamb spent displaying each state behaviour was calculated and analyzed.

The ear-related behaviour of observer lambs was scored for 30 s, halfway through each recording period (i.e. at 15 min before docking and at 15 min after docking) (Table 15). The post-docking sample was taken 15 min after docking as this is when the peak frequency of other pain-related behaviours occurs (Lester et al., 1996); thus it was a sensible time to look for ear-related pain behaviours. A sample duration of 30 s was chosen based on previous studies looking at ear posture (Boissy et al., 2011; Reefmann et al., 2012). Six out of 50 observer lambs were out of view for the whole duration of one of the ear behaviour sampling periods. This meant that their data for both the pre- and post-docking periods were excluded from the analysis (final $n=44$, Table 16b).

Table 15: Behaviours scored for observer lambs. Pain-related behaviours based on Molony and Kent (1997).

Behaviour	Description
Ear behaviours	
Ears Plane	Both ears are perpendicular to the head-rump axis. This is often also associated with the ear auricle facing down.
Ears Forward	Both ears are positioned forward of the perpendicular. This is often also associated with the ear auricles facing forward.
Ears Backward	Both ears are positioned backward of the perpendicular. The ear auricles are not visible from the front.
Ears Asymmetrical	The left and right ear are positioned differently from one another, in one of the other three postures described above.
Ear change	The change of ear position from one of the above to another
Visual Behaviours	
Looking at Ewe	Head and eyes turned in the direction of the ewe
Looking at Actor Lamb	Head and eyes turned in the direction of the actor lamb
Looking at Own Tail	Head and eyes turned in the direction of its own tail
Pain-related Behaviours	
Normal Upright	Standing, walking or playing while exhibiting a usual posture or gait; smooth movements
Abnormal Upright	Standing exhibiting unusual posture e.g. rounded, hunched appearance; ataxia; jerky movements; swaying; walking unsteadily; walking backwards; walking on knees
Normal Lying	Ventral recumbency, all legs tucked under body or very close to body
Abnormal Lying	Twisted lying: ventral recumbency with forelimbs tucked under body, one or both hind limbs partially or fully extended; including dog sitting
Lateral Lying	Lateral recumbency with one shoulder on ground, hind limbs and/or forelimbs fully extended
Repetitive Standing	Number of times lamb moved between lying and standing or part way thereof.
Kick	While lying or standing, rapid extension of one or both hind limbs either away from or towards the body
Roll	Moving from lateral recumbency on one flank to the other. Rolls from flank to dorsum and back to same flank also counted as one event
Jump	All four feet off ground simultaneously, including jumping off hind limbs with forelimbs on pen walls
Stamp	Rapid forceful downward movement of fore or hind foot
Rump Wag	Wag of rump to shift weight, with or without wag of tail
Lip curl/Neck arch	While lying, curling upper lip (like flehmen) with or without arching of head back over the neck
Head Shake	Forceful voluntary shake of head
Head-butt	Forcefully shoving head into another lamb or object or performing action without contact

Table 16: The final number of male, female, singleton and twin observer lambs in each treatment group (FR= Familiar/related, FU= Familiar/unrelated, UU= Unfamiliar/unrelated) included in the quantitative analysis of visual and pain-related behaviours (a), and ear behaviours (b).

a	Treatment group	Sex	Singletons	Twins	TOTAL
	FR	M	0	7	7
		F	0	7	7
	FU	M	6	3	9
		F	7	2	9
	UU	M	6	3	9
		F	3	3	6
	TOTAL		23	25	47

b	Treatment group	Sex	Singletons	Twins	TOTAL
	FR	M	0	6	6
		F	0	7	7
	FU	M	4	3	7
		F	7	2	9
	UU	M	6	3	9
		F	4	2	6
	TOTAL		22	23	44

Videos were scored in a random order and the video observer was semi-blind to treatment group and recording period. Because the camera also partially captured the dams and any siblings in the background (two dams would have denoted either a FU or UU treatment group as opposed to a FR group) and because of the obvious behaviour of the actor lamb in the post-docking period, the video observer may have been able to deduce the treatment group and period.

Qualitative Analysis of Observer Lambs' Behaviour

Video clips for the Qualitative Behavioural Analysis were made from videos of the second round of docking. In this round observer lambs had experienced docking themselves (round one) and therefore may have had stronger behavioural responses to the actor lamb's behaviour. Videos were edited to produce two 1 min clips from each of the pre- and post-docking periods. In each period clips were selected from a 10 min period from 15 min to 25 min through. In order to minimize potential bias in selecting clips, one of the two clips in each period was selected to represent high levels of activity and interaction between the actor and observer lamb, and the other represented low levels of activity between the pair. 'Activity' was defined as close proximity or physical contact between the lambs as well as behaviours including, but not limited to, olfactory investigation and head-butting.

If both lambs were not clearly visible during the designated time period, the video (pre- and post-docking) was not used to generate a clip. This resulted in 14 lamb videos (out of a possible 25 for round two, Table 18) being used to generate 56 clips used for scoring (14 lambs \times 2 periods \times 2 activity levels). Two additional video clips were made from footage outside the 15 to 25 minute time period. These were used as practice clips in Phase One (see below) and were not scored as part of this study. A further two video clips were made from video footage found online (YouTube, LLC URL: <https://www.youtube.com/watch?v=gvcKf9ZU-Iw>, URL: <https://www.youtube.com/watch?v=ren--78GoG4>). These were videos of lambs displaying playful behaviour. The extra 'play' clips were only used in Phase One for term-generation and were not scored as part of the study.

Qualitative Behavioural Assessment procedures

The qualitative assessment component of the study was approved by Massey University Human Ethics Committee (Protocol 12/33). The human observer group comprised 11 undergraduate and postgraduate students (female $n=9$, male $n=2$) who were studying veterinary science, agricultural science or ecology. All had experience observing animals and four had experience observing sheep. None were familiar with qualitative behavioural assessment.

Qualitative assessment occurred in two phases, consistent with Free Choice Profiling methodology (Wemelsfelder et al., 2001). Phase One occurred over one session and consisted of generating the terms the human observers would later use to score the video clips. The observers were told they were taking part in a study to assess the change in a lamb's body language when in the presence of another lamb experiencing pain. They were not told of the allocation of lambs to different treatment groups, nor were they told which clip related to the pre- or post-docking period. Observers were given specific instructions about generating terms to best describe the lambs' behaviour according to Wemelsfelder (2000). They met all together in a lecture room and viewed the video clips on a projector screen. They were all first shown the two practice clips and asked to brainstorm terms together. They were then shown 18 video clips (16 clips which were also scored in Phase Two and two play clips), one at a time, each followed by a 2 min break. In the 2 min break they were asked to write down the terms they thought best described the lamb's behaviour. This part was done without discussion between observers so that each individual generated their own set of terms.

Phase Two consisted of scoring the behaviour of the lambs. Observers each used their own personal terms generated in Phase One. Because of the large number of clips, Phase two occurred over three sessions (session 2= 21 clips, session 3= 21 clips, session 4= 14 clips). They were all shown the video clips, one at a time, each followed by a 4 min break. The clip order was mixed so that the clips reflected a variety of periods (pre- or post-docking), activity levels (high or low) and treatment (FR, FU, UU), with dissimilar clips

after each other. This created contrast between the clips in order to facilitate attention and scoring. In each session, observers were provided with a list of their own terms accompanied by a visual analogue scale (0- 125mm). Sixteen of the clips were the same as in Phase One, however the order of clips was changed and other, previously unseen, clips were also included. Observers were instructed to score the behaviour of each lamb on each of their own terms. If the expression of a term was not seen, a score of 0 was given. Specific details of this method can be found in Wemelsfelder (2000).

Eleven data matrices (one for each human observer, for each of the 56 clips) were obtained for analysis by measuring the distance (in mm) from the '0' end of the visual analogue scale to the point where their tick crossed the line (minimum score= 0, maximum score= 125).

Statistical analyses

Statistical analysis of quantitative data

Observer lambs did not exhibit abnormal lying, abnormal upright, lateral lying, kick, roll, or repetitive standing behaviours and hence these were excluded from analysis.

All statistical analyses were performed using SAS Version 9.2 (SAS Institute Inc., North Carolina, USA). Pre/post (period) data could not be transformed to satisfy assumptions for parametric analyses, therefore analyses were performed on ranked data for ear changes, ears asymmetrical, ears backward, ears forward, ears plane, looking at ewe, looking at other (actor) lamb, looking at own tail, head-butt, normal lying, and normal upright. lip curl, headshake, jump, stamp, and wag occurred infrequently in observer lambs and the frequencies of these behaviours were summed to create a variable named "active behaviours"; these data were not ranked because the distribution was appropriate for parametric analysis.

A MIXED model was used to evaluate the effects of tail docking on pain-related behaviour of observer lambs, with period as the repeated measure, lamb as the random effect and treatment (FR, FU, UU), round (1, 2) and pair-sex as fixed effects.

As well as analyzing ranked data from the pre- and post-docking periods, the change in each behaviour after docking (post – pre) was calculated and analyzed. Because we were interested in the effects of independent variables on the relative magnitude of change due to docking, change variables were calculated and then ranked. Ranked change data for all behaviours except active behaviours were analyzed using an ANOVA with treatment, round and pair-sex as independent variables. The data for "active behaviours" were not ranked because the distribution was appropriate for parametric analysis. When significant effects were found, post hoc tests were performed using Bonferroni adjustment for multiple comparisons.

Statistical analysis of qualitative data

Full details of the statistical procedure can be found in Wemelsfelder (2000). All preliminary analyses were performed using GenStat 11.1 (VSN International).

Generalized Procrustes Analysis (GPA) was used to analyze the data. A Student's t-test was used to determine whether the consensus profile was significantly different from a mean randomized profile. A significant difference indicates that the consensus profile was a meaningful feature of the data and not a statistical artefact. Principal Component Analysis (PCA) was then used to reduce the number of dimensions of the consensus profile to best explain the majority of variation between the lambs. Each lamb received a score on each of the consensus dimensions, which were used for further statistical analysis (below). Three-dimensional Word Charts (examples provided in Figure 10) were made by calculating how the co-ordinates of the consensus profile and each human observer's data matrices correlated. By looking at each individual's word chart, the axes of the consensus profile could be defined. This was done by looking for semantic similarity between the terms on the human observers' word charts that had the highest positive and negative correlations with each of the dimensions on the consensus profile (Table 17). This information was then summarized into two labels for each of the consensus dimensions. Therefore the labels are not interpretations made by the researcher, but rather, the words used to label the axes were generated by the human observers, who were blind to the study purpose and design.

A MIXED model was used to evaluate the effects of tail docking on people's qualitative assessment of an observer lamb's behaviour, with period as the repeated measure, lamb as the random effect, and treatment (FR, FU, UU), observer sex, actor sex, and activity level (high, low) as fixed effects.

The qualitative data represented a sub-set of the quantitative data (14 lambs selected from a possible 25 in round two of docking). Because of the low lamb numbers in this subset not all combinations of actor and observer sex (pair-sex) were represented in each treatment group thus observer and actor sex were included as separate factors instead (Table 18). When significant effects were found, post hoc tests were performed using Bonferroni adjustment for multiple comparisons. Differences were considered significant at $P < 0.05$.

Table 17: Human observers' (n = 11) terms showing the highest positive and negative correlation with the axes of dimensions one, two and three of the consensus profile. Numbers in brackets denote the number of human observers who generated this term. If there is no number then only one person generated this term.

Dimension	Positive	Negative
1	Playful (3), alert (3), active (2), aggressive, adventurous, distressed, dominant, attentive, excited, interested.	Calm (7), relaxed (4), comfortable (2), peaceful (2), docile, content, patient, dull.
2	Aggressive (10), angry (2), agitated, grouchy, forceful, superior, short-tempered, rambunctious, annoyed, losing-temper.	Curious (5), interested (3), inquisitive (2), concerned (2), worried (2), cautious, approachable, intrigued, investigative, cool
3	Caring (3), compassionate (2), dominant, protective, snuggly, comforting, protective, warm, consoling, sympathetic, friendly.	Avoidant, apprehensive, distracted, longing, frightened, uneasy, ignoring, stay-away-ish, wanting-to-escape, distancing, scared, unsure, cautious, avoiding, uncertain.

Table 18: The final number of male and female observer lambs in each treatment group (FR= Familiar Related, FU= Familiar Unrelated, UU= Unfamiliar Unrelated) included in the **qualitative analysis**.

Treatment group	Females	Males	TOTAL
FR	2	2	4
FU	3	2	5
UU	0	5	5
TOTAL	5	9	14

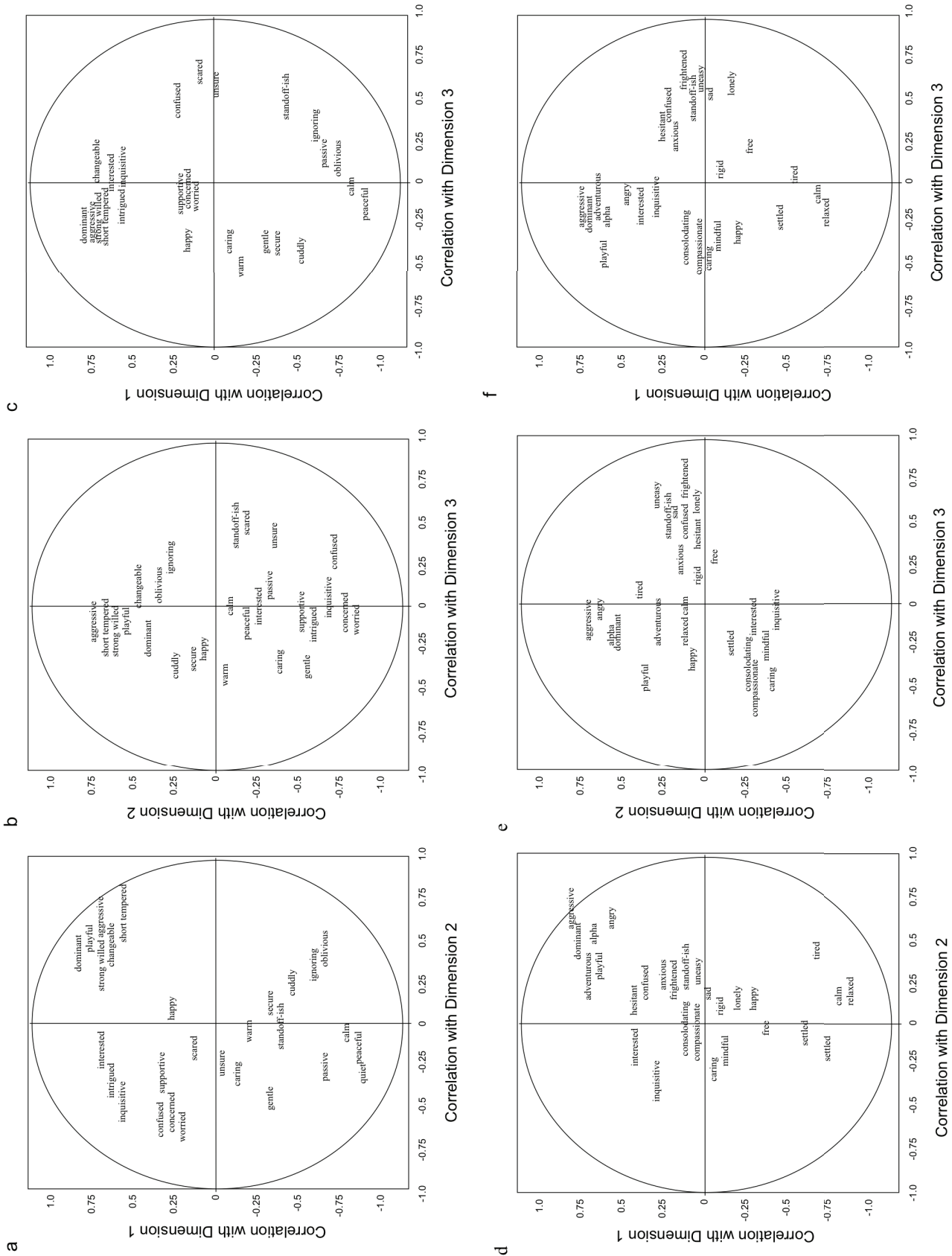


Figure 10: Word chart of observer 7 for dimensions one and two (a), two and three (b), and one and three (c); as well as word chart of observer 2 for dimensions one and two (d), two and three (e), and one and three (f). The word charts of all observers were summarized as: (high to low) dimension one alert/active to docile/calm; dimension two aggressive/agitated to curious/interested; and the dimension three as avoidant/apprehensive to comforting/consoling.

Results

Quantitative data

Effects on repeated measures data

Tail docking of the actor lamb resulted in a significant increase in the frequency of head-butting (Mean ranks \pm SE: pre 41.5 ± 3.8 , post 55.0 ± 3.8) and active behaviours (Raw mean \pm SE: pre 1.6 ± 0.4 , post 3.8 ± 0.4) in the observer lambs regardless of treatment group, round or pair-sex. In addition lambs spent more time looking at the other (actor) lamb (Mean ranks \pm SE: pre 32.0 ± 3.3 , post 64.0 ± 3.3) and with their ears backward (pre 29.3 ± 3.2 , post 58.1 ± 3.2). They spent less time with their ears in the plane posture after docking of the actor lamb (pre 53.1 ± 3.8 , post 37.0 ± 3.8).

Period and round had an interactive effect on the frequency of ear posture changes and the proportion of time spent looking at own tail (Table 19). Lambs changed their ear posture more often (Round 1: pre 28.7 ± 4.7 , post 61.1 ± 4.7 , $t = 5.55$, $P < 0.0001$; Round 2: pre 40.7 ± 5.0 , post 43.3 ± 5.0) and looked at their own tail for longer (Round 1: pre 33.2 ± 4.7 , post 57.0 ± 4.7 , $t = 4.65$, $P < 0.0001$; Round 2: pre 49.5 ± 4.9 , post 48.8 ± 4.9) after the actor was docked in round 1, but not in round 2.

Period and treatment had an interactive effect on the proportion of time spent looking at own tail (Table 19). Only FR observer lambs spent more time looking at their own tail after the actor was docked (FR: pre 36.4 ± 6.2 , post 64.2 ± 6.2 , $t = 3.99$, $P = 0.003$).

Pair-sex had an effect on the frequency of ear posture changes and Active Behaviours (Table 19), however after adjustment for multiple comparisons no significant differences among pair-sexes were found.

Effects on change data

Observer Lambs in round 1 showed a greater increase in the frequency of ear posture changes (Mean ranks \pm SE: Round 1 27.3 ± 2.5 , Round 2 16.1 ± 2.6 , $F_{1,43} = 9.68$, $P = 0.003$) and spent more time looking at their own tail (Round 1 29.6 ± 2.8 , Round 2 19.4 ± 2.9 , $F_{1,46} = 5.96$, $P = 0.02$) compared to lambs in round 2.

FR lambs tended to show a greater increase in looking at their own tail than UU lambs (FR 33.5 ± 3.0 , UU 19.1 ± 3.0 , $F_{2,46} = 2.68$, $P = 0.09$)

Table 19: Results of MIXED model analysis on **quantitative data** from observer lambs before and after actor tail docking (period). Active Behaviours are the sum of the frequencies of Lipcurl, Headshake, Jump, Stamp, and Wag. Factors included in the model are pair-sex (same-sex female, same-sex male, mixed-sex observer female, mixed-sex observer male), round (1, 2) and treatment (Familiar Related, Familiar Unrelated, Unfamiliar Unrelated, Unfamiliar Related). Bold text denotes a statistically significant result at $P < 0.05$.

Behaviour	Period		Pair-sex		Round		Treatment		Interactive Effects		
	F _(1,40)	p	F _(3,39)	p	F _(1,29)	p	F _(2,29)	p	Type	F	p
Visual Behaviours											
Looking at Ewe	3.40	0.07	1.45	0.25	3.67	0.07	1.00	0.38			
Looking at Lamb	48.39	<0.0001	1.82	0.17	0.04	0.84	0.35	0.71			
Looking at Own Tail	9.72	0.003	0.50	0.68	0.45	0.51	0.30	0.74	Period*Round	11.37	<0.01
									Period*Treatment	4.93	0.01
Ear Behaviours											
Ears Asymmetrical	0.69	0.41	0.23	0.87	0.20	0.65	0.67	0.52			
Ears Back	31.36	<0.0001	2.06	0.12	0.03	0.86	0.15	0.87			
Ears Forward	2.06	0.16	1.89	0.15	0.00	1.00	0.00	1.00			
Ears Plane	11.34	0.002	0.67	0.58	0.02	0.88	0.11	0.90			
Ear Changes	19.12*	<0.0001	2.94	0.05	0.63	0.43	0.55	0.58	Period*Round	10.40	<0.0001
Pain-related Behaviours											
Normal Upright	0.00	0.98	0.80	0.50	2.05	0.16	0.25	0.78			
Normal Lying	0.03	0.87	0.85	0.48	2.14	0.15	0.23	0.80			
Headbutt	13.02	0.001	0.64	0.60	0.02	0.89	2.07	0.15			
Active Behaviours	16.38	<0.0001	3.81	0.02	1.65	0.21	1.06	0.36			

*df = 1,42

Table 20: Results of MIXED model on **qualitative data** from observer lambs before and after actor tail docking (period). Factors included in the model are treatment (Familiar Related, Familiar Unrelated, Unfamiliar Unrelated), observer sex (male, female), actor sex (male, female), activity (high interaction, low interaction). Bold text denotes a statistically significant result at $P < 0.05$.

Dimension	Period		Treatment		Observer Sex		Actor Sex		Activity		Interactive Effects	
	F _(1,13)	P	F _(2,9)	P	F _(1,9)	P	F _(1,9)	P	F _(1,13)	P	Type	F _(df)
Dimension 1	13.11	0.01	3.34	0.08	2.99	0.12	0.32	0.58	7.01	0.02	Period*Observer Sex	10.20 _(1,14)
Dimension 2	0.70	0.42	1.28	0.32	1.84	0.21	0.14	0.72	0.28	0.61	Period*Treatment	4.08 _(2,10)
Dimension 3	2.07	0.17	3.44	0.08	0.1	0.75	0.82	0.39	4.27	0.06	Treatment*Activity	7.70 _(2,11)

Results of qualitative data

The consensus profile explained 55.16% of the variation among data matrices (Procrustes statistic = 55.16). This differed significantly from the randomized profiles (one-tailed Student's *t*-test, *df* = 99, *t* = 60.37, *P* < 0.001). The three dimensions of the consensus profile explain 36.8%, 13.3% and 9.1% of the variation among lambs, respectively. The 11 word charts were similar semantically (Table 17). The charts summarized the first dimension as (high to low) alert/active to docile/calm; the second as aggressive/agitated to curious/interested; and the third dimension as avoidant/apprehensive to comforting/consoling.

Dimension One: alert/active to docile/calm

Activity level affected the dimension one scores (Table 20). Observer lambs in high activity clips were rated as significantly more alert and active than in low clips (Means \pm SE: high 0.00 ± 0.01 , low -0.02 ± 0.01).

Period and sex of the observer had an interactive effect on dimension one scores (Table 20). Female observer lambs were rated as significantly more alert and active in the post-docking period compared to the pre-docking period (Figure 11). There were no significant changes in dimension one scores for males from pre- to post-docking. In addition, before docking female observer lambs were rated as more docile and calm than male observer lambs, who were more alert and active.

Period and treatment also had an interactive effect on dimension one scores (Table 20). However, after adjustment for multiple comparisons no significant differences among treatment groups were found for any period. There was a tendency for UU observer lambs to be rated as more alert and active in the post-docking period compared to the pre-docking period (Figure 12). There was also a tendency for FU observer lambs to be rated as more alert and active than FR observer lambs in the pre-period.

Dimension Two: aggressive/agitated to curious/interested

No effect of period, treatment, observer sex, actor sex, activity level or any interaction effects were found on dimension two scores (Table 20).

Dimension Three: avoidant/apprehensive to comforting/consoling

Treatment and activity had an interactive effect on dimension three scores (Table 20). FR observer lambs in high activity clips were seen as more comforting and consoling compared to FR observer lambs in low activity clips and as compared to FU observer lambs in high activity clips (Figure 13).

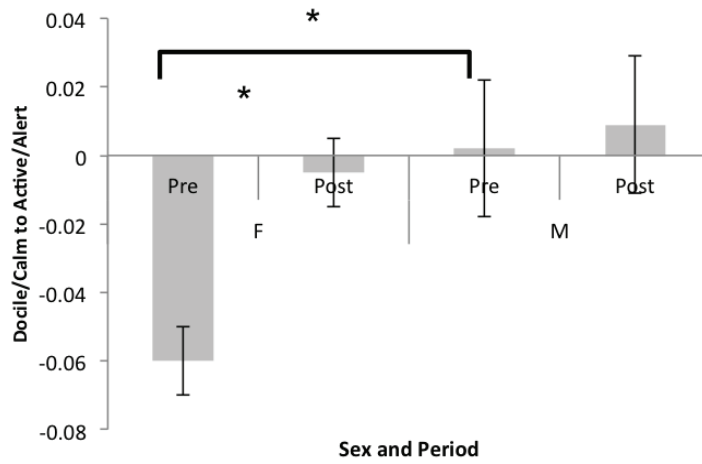


Figure 11: Mean \pm SE (sex*period interactive effect) dimension one scores. F = female, M = male. Pre = before docking, post= after docking. Significant differences at $P < 0.05$ are denoted by an asterisk (*).

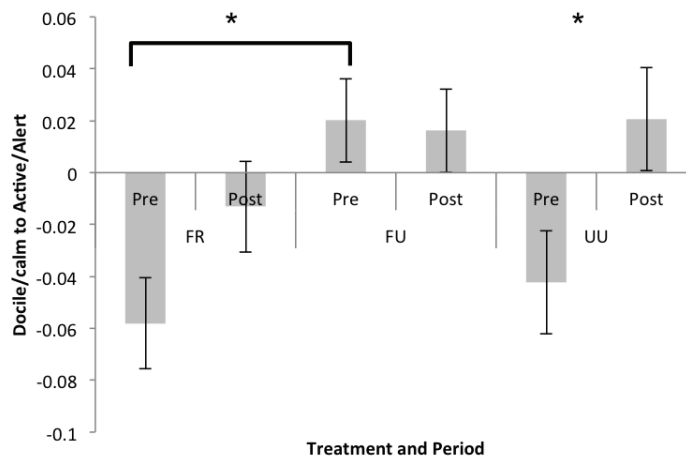


Figure 12: Mean \pm SE (treatment*period) dimension one scores. FR = Familiar Related, FU = Familiar Unrelated, UU = Unfamiliar Unrelated. Pre = before docking, post= after docking. Tendencies for differences at $P < 0.1$ are denoted by an asterisk (*).

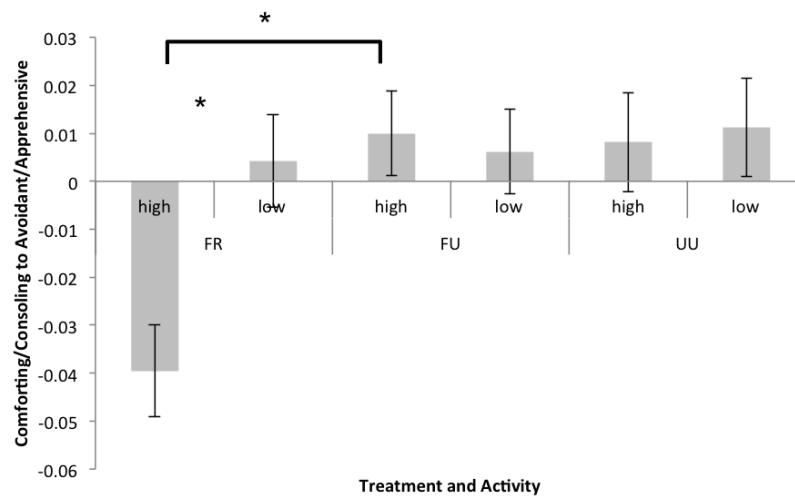


Figure 13: Mean \pm SE (treatment*activity) dimension three scores. FR = Familiar Related, FU = Familiar Unrelated, UU = Unfamiliar Unrelated. Significant differences at $P < 0.05$ are denoted by an asterisk (*).

Discussion

The aims of this study were to use both quantitative and qualitative behavioural assessment techniques to investigate whether lambs display socially facilitated behaviour (SFB) indicative of empathy when in the presence of a conspecific in pain and to explore how factors of familiarity, relatedness, sex and previous experience of pain affect the expression of SFB. in this context.

Discussion of quantitative data

We found some evidence to support the idea that observer lambs changed their behaviour in a way that might reflect empathy for conspecifics in pain. After docking of the actor lamb, twin observer lambs spent more time looking at their own tails, and all observer lambs spent more time looking at the actor, showed ear posture changes consistent with experiencing a negative emotion and showed an increase in active behaviour.

We may expect that related observers or those that are familiar with the actor would be more likely to show socially facilitated behaviour because doing so may provide an evolutionary fitness advantage to the observer (Hamilton, 1964; Langford et al., 2010). We saw some difference in response to conspecific docking according to presumed familiarity or relatedness. Familiar related observers spent more time looking at their own tail after their sibling was docked, whereas there was no change in this behaviour for familiar or unrelated lambs. Similar to previous studies of SFB associated with pain (e.g. Langford et al., 2006) we found that observer lambs were mimicking or engaging in some of the same pain-related behaviours as the actor lamb including looking at its own tail. Not only did they mirror the type of behaviour, but also the intensity. Actor lambs in round 1 spent more time looking at their own tail (Chapter 3.2) and so too did observers. This suggests that a difference in intensity of the actors' behaviour influences the intensity of the observers' behaviour potentially as well as the intensity of the empathy experience.

Observers also showed signs that they were distressed by the presence of a conspecific in pain. Observer lambs showed an increase in time spent with their ears back, a decrease in time spent with their ears plane and a greater frequency of ear posture changes in round 1. We have previously demonstrated that this pattern of ear-related behaviours is associated with the negative emotional experience associated with pain in lambs (Chapter 2.1). A change in ears plane and the number of ear posture changes are also supported by other studies of adult sheep (Boissy et al., 2011; Reefmann et al., 2009a; Reefmann et al., 2009b; Stubbsjoen et al., 2009). Again, the greater frequency of ear posture changes in round 1 may reflect a more intense empathic experience by observer lambs. Alternatively, the novelty of the actor lambs' behaviour in round 1 may be underlying this effect.

As well as mimicking the behaviour of looking at own tail, observer lambs also displayed an increase in active behaviours after actor docking including lip curling, head shaking,

jumping, stamping and tail wagging. This finding is interesting, as displaying active behaviours in response to conspecific pain may be inappropriate for a prey species such as sheep. Displaying pain-related behaviours increases the risk of alerting potential predators to an animal's weakened condition (Williams, 2002). However, observer lambs also initiated and displayed head-butting behaviour which could relate to a desire to remove the novel stimulus, therefore minimizing unwanted attention towards itself. Alternatively, the change in behaviour displayed by the observer may be a result of the increased activity or abnormal behaviour of the actor (Chapter 3.2).

Discussion of qualitative data

This is the first study to use the free choice profiling (FCP) technique to generate descriptive terminologies for lamb social behaviour in the context of pain. Using QBA we found evidence that observer lambs were affected by seeing a conspecific in pain, resulting in differences in behavioural expression. However, this effect varied according to the relationship between the actor and observer.

The presumed familiarity and relatedness of lamb pairs affected how observer lambs were perceived overall, regardless of whether the actor had been docked or not. A high level of interaction between twin lambs was seen as being comforting or consoling in nature, compared to a similar level of interaction between familiar, yet unrelated lambs.

Presumed familiarity and relatedness also affected how people described the response of the observer to conspecific docking. Only UU lambs tended to be seen as more alert and active after docking compared to before. Unfamiliar lambs may represent a social threat (Lynch et al., 1992), so that when the actor begins to behave in a novel and potentially distressing manner, the observer reacts with an alert or active response. Lambs that were related were seen as more docile or calm before docking compared to familiar, but unrelated lambs and did not become systematically more active/alert after actor docking perhaps because they spent the most time together before being put into the test environment and therefore were calmer overall.

There is evidence that twin lambs behave differently in the test environment than other groups (Chapter 3.2). This may have to do with twins being reared in a different social environment than familiar lambs. Twins spend more time together due to feeding from the same dam (Walser and Williams, 1986), compared to familiar lambs who are raised in the same paddock and may only encounter each other occasionally.

The sex of the observer lamb also influenced people's perception of how alert/active or docile/calm they were. Female observers were perceived as more calm than males in the pre-docking period however became more active and alert after docking. Sex differences in play behaviour (Lynch et al., 1992) in the pre-docking period may have obscured any change in behaviour after docking. Male lambs engage in more active and aggressive play than do females (Sachs and Harris, 1978), meaning they may have been more active than females in the pre-docking period. After docking, both males and females were more likely to be paying attention to their test-mate in pain, and as a result display more active behaviour. Because of the high level of activity that males displayed in the pre-docking period, human observers may not have seen a noticeable change in behavioural expression in male lambs.

The level of interaction between the lambs also influenced people's perception of their behavioural expression. When lambs were showing high levels of interaction within the pair as well as closer proximity, they were rated both as more active and alert (all lambs) and twins were rated as more compassionate and consoling. This makes intuitive sense, and highlights that even subtle differences in behaviour between the same lambs can alter people's perception of their emotional state.

Interestingly, there were no differences according to period, treatment, sex or previous experience on dimension 2 (aggressive/curious). This may be because these terms described reactive behaviours, rather than emotions or traits that would persist long enough for there to be differences across treatment or sexes. In other words, all observer lambs may have reacted aggressively or curiously at some stage to the actor, but different groups were not more or less aggressive or curious than others.

General discussion

Both quantitative and qualitative measures provided some evidence that observer lambs displayed socially facilitated behaviour (SFB) when in the presence of a conspecific in pain and that doing so caused a negative emotional state in observers. After actor docking, observers showed behavioural changes similar to those demonstrated by actors, in particular changes in ear posture and looking at their tail. In addition, familiar related lambs looked at their tail more after actor docking than other groups and were rated as more comforting/consoling, which is consistent with predictions of SFB.

These findings provide some evidence that observer lambs may have been experiencing empathy at a primal level (Edgar et al., 2012; Panksepp and Panksepp, 2013). Empathy as defined here refers to the shared emotional state between two individuals, which leads to the generation of an appropriate action (Preston and de Waal, 2002). In previous animal studies as well as this one, empathy in observers to conspecific pain has been accompanied by a display of behaviours similar to those demonstrated by the actor i.e. socially facilitated behaviour (Atsak et al., 2011; Langford et al., 2006; Langford et al., 2010; Watanabe, 2012).

The ability to experience empathy may be useful for facilitating successful interaction between social individuals by being able to understand another and predict their actions (Singer, 2006).

There was some agreement between quantitative and qualitative measures of SFB and empathy but no strong consistency. We may have expected a period*treatment effect for dimension three where twin lambs were rated as more comforting and consoling after actor docking than before. This may have indicated that the experience of empathy was causing lambs to display pro-social behaviour, as has been previously found in rats (Langford et al., 2010, Watanabe, 2012). It is perhaps surprising that there were no treatment differences using QBA for dimension 2 (aggressive/curious). The lack of consistency may also be to do with the types of behaviours that were shown by observer lambs. Ear posture changes and looking at the tail are fairly subtle, and so human observers doing QBA may not have picked up on these when viewing the lambs' body language overall. In addition, while the consensus among human observers was highly significant, the three dimensions only explained 59.2% of the variation in human observers' descriptions of lamb behaviour. This lower than most other studies using QBA for assessment of sheep expression (53% (Wickham et al., 2012) and 78% to 90% (Phythian et al., 2013)). None the less, QBA provided a unique insight into lamb social behaviour and the descriptors generated are interesting in themselves.

A potential limitation to the current study is that the selection of clips may have inadvertently biased human observer's perception of FR lambs. The quality of interaction between twins may be different from the type of interaction occurring between other pairs. For example greater social contact between twins in the form of lying together may be interpreted as comforting or consoling by human observers.

Conclusion

This is the first study to demonstrate SFB of lambs, possibly indicative of empathy, in response to conspecific pain. The use of a Free Choice Profiling technique offered a complementary approach to describing lamb social behaviour by evaluating behavioural and emotional expression in terms of the whole animal, rather than specific behaviours. However in this case quantitative measures seemed better able to detect differences in more subtle observer lamb behaviours such as ear changes and looking at their own tail according to the relationship between the actor and observer lamb and novelty of the test situation.

Literature review



Animals that alter their behaviour based on observations of their environment, including observations of pain behaviour, are likely to enhance their survival or reproductive success. Thus we would expect conspecifics to alter their behaviour when viewing another individual in pain and there is evidence from various mammalian species that this occurs. Likewise, there is evidence that the presence of a conspecific can influence the perception and expression of pain. However, this interaction between individuals appears to depend on the genetic and social relationship between them. The social influences on pain perception and expression have only just begun to be investigated and the studies contained in this thesis add a great deal to this research area. Therefore this literature review will make reference to the findings of the previous experimental chapters of this thesis.

Evidence for a social function of pain-related behaviour

5

This chapter is based on the following paper:

Guesgen, M.J., Beausoleil, N.J., Minot, E.O., Stewart, M., Stafford, K.J. Evidence for a social function of pain-related behaviour. PLoS ONE (in preparation).

Abstract

Pain is a phenomenon that has not only physical, but also emotional aspects. There is growing evidence to support the idea that emotional neural systems in the brain drive future behaviour. Physiological changes associated with pain depend on the limbic system, and are largely similar across different mammalian species. However the complex behavioural reactions accompanying the pain experience also involve the cerebrum, a structure that varies greatly across species and therefore produces a range of different reactions. The diversity of pain-related behaviours suggests multiple functions. Certain behaviours, such as changes in ear posture or facial expression appear to have a different, i.e. social, function to other pain-related behaviours that may function to assist in alleviating pain, for example abnormal lying. Pain behaviour may also be valuable or dangerous in that it is observable by other animals, thus providing access to information about the emotional state of the animal. This review discusses the proposed functions of pain-related behaviour from an evolutionary viewpoint. The main focus of this review will be on a social function of pain-related behaviour. However, other non-social functions of pain behaviour, namely avoidance and healing, will also be discussed. We will demonstrate how viewing pain expressed by another can alter the behaviour and emotional state of an observer animal, as well as how the presence of conspecifics can influence the pain experience of an individual. Throughout this review, we will emphasize how the interaction between individuals depends on the genetic and social relationship between them. We will present empathy as the main mechanism underlying changes in social pain-related behaviour and discuss how empathy has, and can be, measured in non-human mammals.

Keywords: Evolution, Pain, Social

Introduction

Pain has been defined as:

“An aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues. It changes the animal’s physiology and behaviour to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery” (Molony and Kent, 1997),

The definition of pain as having physical and emotional components means that the ability to feel pain requires an animal to fulfill certain criteria. Firstly, they must be sentient, or have the ability to experience positive and negative affective (emotional) states (Duncan, 2006). Secondly, the animal must be in a state of consciousness; in very basic terms, aware of its surroundings (Mellor and Diesch, 2006). Currently, all vertebrates as well as cephalopods are considered able to experience something analogous to human pain based on the above factors (Smith, 1991). This review will be restricted to mammals.

Pain processing then is initiated when peripheral receptors, also called nociceptors, are activated by noxious stimuli (Grubb, 1998). These fibres generate action potentials in response to stimuli such as extreme temperature, stretch or chemical stimulation from damaged cells (Grubb, 1998). Action potentials travel along afferent axons and terminate in the superficial and deep dorsal horn of the spinal cord. Within the spinal cord, activated fibres release a variety of neurotransmitters, in particular glutamate, which stimulates an excitatory response in the spinal neurons which then relay the pain signal to the brain (Dingledine et al., 1999; Vinuela-Fernandez et al., 2007).

The processes described above refers to the physiology underlying nociception, or the ability to sense noxious stimuli (Woolf and Ma, 2007). However, nociception is only a part of the pain experience. The perception of pain requires both cognitive and affective (emotional) components (Kavaliers, 1988), which involve processing of the nociceptive signal by the brain. The areas of the brain required for cognitive and affective interpretation of incoming signals are the limbic system and cerebrum (Bruce and Neary, 1995).

The limbic system and cerebrum also initiate particular physiological and behavioural responses to pain. For example, the hypothalamus and pituitary gland stimulate the release of opioids which produce analgesia (pain relief) or of glucocorticoids which cause an anti-inflammatory response at the site of nociceptor stimulation (Bruce and Neary, 1995). Similarly, adrenaline and noradrenaline are released by sympathetic nerve fibres and the adrenal medulla via stimulation from the autonomic nervous system which results in physiological changes associated with pain such pupil dilation, changes in peripheral blood flow, quality of respiration, and heart rate (Molony and Kent, 1997; Rutherford, 2002).

The cerebrum is responsible for a variety of functions including: initiation of movement, learning and memory as well as communication (Fitzgerald and Anand, 1993). Activation of the cerebrum for pain processing can generate motor patterns, or behaviour in response to noxious stimulation (Anand et al., 2000). The behavioural reactions accompanying the pain experience are of particular interest, because they may be accessible to other animals in the environment.

While previously there has been trepidation in ascribing emotions to non-human mammals, there is growing evidence to support the idea that neural systems in the brain do not just control behaviour but also generate emotional states that can guide future behaviour (Panksepp, 2011; Panksepp and Panksepp, 2013). These emotional states precede and drive behaviour. It is important to note, that Panksepp's use of the term 'emotion' is different to what we may intuitively think of as an emotion, in terms of mood or temperament. He classifies emotions as primary-process brain systems, in other words, systems that underlie outward expression. Therefore, under this view, all behaviours are driven by emotion and emotions are fundamental and integrally linked with brain systems. For example, the emotion of fear occurs through activation of the amygdala, ventral and dorsal periaqueductal grey matter, hypothalamus and ventral medial forebrain bundle (Panksepp and Panksepp, 2013). Corticotrophin releasing factor then mediates neural circuits underlying behaviours which may reduce the likelihood of destruction by predators, for example. In contrast, activation of similar brain areas but with the involvement of oxytocin or vasopressin may result in an emotion of lust, which drives sexual behaviours (Panksepp and Panksepp, 2013). The key aspect of Panksepp's work is a subcortical system that evolved before the evolution of the human neocortex and is consistent across all mammals.

This review discusses the proposed functions of pain-related behaviour from both proximate and ultimate viewpoints. A proximate view includes the immediate biological and environmental factors that influence pain-behaviour expression, whereas an ultimate view explores how traits may have evolved (Tinbergen, 1963). The main focus will be on a social function of pain-related behaviour. However, other non-social functions of pain behaviour will also be discussed.

I will first explain the types of pain-related behaviour (In the section "Characterizing pain-related behaviour"), then discuss the two non-social functions of pain behaviour: avoidance and healing. The remainder of the review will investigate the social function of pain behaviour by looking at how viewing pain expressed by another can alter the behaviour and emotional state of an observer animal (section "Socially Facilitated Behaviour") as well as how the presence of conspecifics can influence the pain experience of an individual (section "Social Buffering"). Throughout this review, we will emphasize how the interaction between individuals appears to depend on the genetic and social relationship between them, as well as briefly highlighting how sex and previous experience of pain may also alter pain-related social interactions (section "Other factors influencing the social function of pain behaviour").

We will present empathy as the main mechanism underlying changes in social pain-related behaviour (section “Mechanisms underlying Socially Facilitated Behaviour and Social Buffering”) as well as the alternative Imitation Theory, and discuss how empathy has, and can be, measured in non-human mammals.

Characterizing Pain-related Behaviour

Behavioural responses to pain may include reflexive behaviours, such as limb withdrawal. Reflex responses are rapid, automatic responses mediated by circuits involving the spinal cord and lower brain regions and do not require conscious thought (Konorski, 1948). Therefore, this review will not focus on pain assessment techniques based on reflex responses, such as the tail-flick test or limb withdrawal (D’Amour and Smith, 1941) or the finch-jump test (Evans, 1961). Most behavioural responses to pain are complex behaviours including: attempts to avoid or escape from the noxious stimulus (Allen, 2004) increased or altered vocalization (Weary and Fraser, 1995), abnormal postures or altered locomotion (Molony and Kent, 1997), and decreased in time spent performing normal behaviours (Hassall et al., 1993).

Changes in facial expression may also accompany the pain experience. In humans, the facial expression for pain has been characterized according to the Facial Action Coding System and scores correlate with self-reports of pain (Ekman and Friesen, 1978). The human pain face includes lowering of the eyebrows, raising of the cheeks, squinting of the eyes, wrinkling of the nose, and raising of the upper lip (Ekman and Friesen, 1978). Similar coding systems have also been developed for mice (Langford et al., 2010a), rats (Sotocinal et al., 2011), and rabbits (Keating et al., 2012). These animals show common changes in facial expression when experiencing pain: eye squinting, nose bulge, cheek bulge (mice) or flattening (rats and rabbits), pushing ears back and flattening of whisker pads. Research suggests that sheep also show a measurable facial expression for pain. Pain in lambs may be accompanied by eye squinting, tightening of the lips and mouth, pointing of the nose, and cheek flattening however this needs to be validated (Chapter 2.2). Sheep also show ear-related changes in response to pain, namely a greater number of ear posture changes, spending more time with their ears backward and less time with their ears in a horizontal position (Figure 2, Chapter 2.1).

The diversity of pain-related behaviours suggests multiple functions. Certain behaviours, such as changes in ear posture or facial expression appear to have a different function to other pain-related behaviours (for example an increase in abnormal lying) that may function to assist in alleviating pain (Allen, 2004). Thus, different pain behaviours may be more or less protective or communicative. For example, facial expressions such as squinting may be mainly communicative, but also function to protect the eyes (Craig, 2009). Therefore, it should be stated that we do not view the functions described in this review as purely mutually exclusive.

These various behaviours have likely developed under selective pressure, as they give the animal some advantage, be it a quick recovery from injury (healing), or solicitation of help from other individuals or both (Prkachin, 1986; Williams, 2002). However, displaying pain behaviour is a trade-off as it may also potentially alert predators to an animal's weakened condition.

Avoidance function of pain

Pain is aversive by nature and emotionally unpleasant. The perception of pain can act as an alarm that may induce behaviours with the potential to decrease the cause of the pain sensation (Eccleston and Crombez, 1999; Le Bars et al., 2001). Thus pain serves to promote an animal's health and survival (Williams, 2002). From a proximate standpoint, the individual may be able to immediately avoid actual or potential tissue damage by withdrawing the body part or attempting to escape from the situation (Allen, 2004; Bateson, 1991). The neurological basis of thermal pain perception elucidates such responses: The threshold at which nociceptive fibres are activated is lower than the point at which actual tissue damage occurs. Mammalian thermal nociceptors are activated at 38° to 42° Celsius, whereas tissue damage occurs at about 45° Celsius in humans and even higher in other mammals (Morrison et al., 2013, Chapter 3.1). In this way, the pain processing system actually predicts and avoids potential damage (Morrison et al., 2013).

From an ultimate standpoint, individuals that respond appropriately to pain may increase their fitness by avoiding serious injury or reducing their risk of predation (Stearns and Hoekstra, 2005). The sensation of pain should also act as a stimulus for learning to avoid tissue damage in the future (Bateson, 1991; Dunlop et al., 2006; Goubert et al., 2011; Kleinbohl, 2007; Yarali et al., 2008). This requires that the animal learns to associate the aversive sensation of pain with a particular place, event, or stimulus (Bateson, 1991). Avoidance learning involves mechanisms of vigilance to threat (Chapman, 1995), memory, reward pathways (Fordyce, 1976; Smith and Buchanan, 1954), and neural changes (Konorski, 1948; Rogan et al., 2005).

Healing function of pain

In the short-term, certain behaviours can help to reduce the sensation of pain (Fordyce, 1976). For example rubbing or licking a painful body part can activate particular non-nociceptive fibres which interfere with signals in the dorsal horn of the spinal cord, thus 'closing the gate' to the transmission of these signals to the brain (Dickenson, 2002; Wall, 1979).

Furthermore, behaviours such as limping or guarding of the area limit movement and can therefore facilitate healing by limiting further tissue damage or avoiding disrupting newly formed regenerative tissue (Fitzpatrick et al., 2006; Le Bars et al., 2001). Such pain-related behaviours would be adaptive for an animal, as it avoids activities or movements that may delay recovery time (Wall, 1979).

Social function of pain

Pain behaviour may also be valuable or dangerous in that it is observable by other animals, thus providing access to information about the emotional state of the animal. In the simplest terms, animal interactions occur in a dyad, where the animal displaying a particular repertoire of behaviours is the actor and another viewing and potentially responding to the actor's behaviour is the observer.

Both conspecific and heterospecific observers may attend to cue or signal information that alerts them to an animal's condition. Expressing pain to others is valuable as it may elicit help from conspecific observers (Craig and Prkachin, 1978). However it comes with the risk of alerting undesired individuals, for example predators, to the actor's vulnerable state. For the observer, paying attention to another's pain behaviour may provide information about potential threat, allowing the observer to learn how to avoid the stimulus (Craig and Prkachin, 1978; Mateo, 1996). In this way, changes in behaviour brought about by the sensation of pain may act as a cue or signal.

A cue can be described as a feature of the environment (including a conspecific) that can be used by an animal to guide future behaviour (Maynard Smith and Harper, 2003). In contrast, a signal is an act or structure which has evolved for the specific purpose of altering the behaviour of another animal and this evolution has occurred because of the receiver's (observer's) response (Maynard Smith and Harper, 2003). The key distinction between a cue and a signal is that a cue does not necessarily result in a change in behaviour from the observer whereas a signal does and has evolved to do so. For example, an alarm call from an animal is a signal because it has evolved for the purpose of alerting other group members of danger. In contrast, a mammal breathing out carbon dioxide which a mosquito then follows is a cue because the mammal does not exhale carbon dioxide on purpose to attract the mosquito. Pain-related behaviours are likely to be an honest signal (rather than a misleading one such as mimicry), because they carry a potential risk to the animal (Wells, 2003). Thus pain behaviour has likely evolved, in part, because of the responses of conspecific observers.

This review will provide evidence of a social function of pain behaviour in non-human mammals. Animals that alter their behaviour based on observations of their environment, including observations of pain behaviour, are likely to enhance their survival or reproductive success (Stearns and Hoekstra, 2005). Thus we would expect conspecifics to alter their behaviour when viewing another individual in pain and there is evidence from various mammalian species that this occurs. Likewise, there is evidence that the presence of a conspecific can influence the perception and expression of pain.

The likelihood of social interaction, as well as the type of interaction, will depend on the relationship between individuals. While it is advantageous for an individual to increase its own fitness (Stearns and Hoekstra, 2005), it is also advantageous for it to engage in behaviours that promote the survival of those most closely related to it, as these individuals carry 25%-50% of the same genes (Hamilton, 1964). According to evolutionary theory then, communication of pain via behavioural changes is most likely to occur between parents and offspring who share 50% of their genes (Hamilton, 1964). In addition, it is advantageous for offspring to maximize the investment or help they get from their parents in order to increase their fitness (Trivers, 1974). Individuals may also engage with those who are familiar to them as they represent members of their social group and because they are more likely to send signals that are honest and relevant. Although such behaviours may be costly to the individual and group (for example alarm calling may draw attention to the individual or group), they benefit the group and allow them to outcompete other groups (Hamilton, 1964).

In the following sections of this review, we will discuss how the relationship between the actor and observer affects the expression of behaviour. The following sections are summarized in tables 21 and 22. Although the sections regarding the actor and observer are laid out separately, it should be made clear that the interactions between the two are reciprocal. In a sense, interactions occur in a cycle where an actor displays pain behaviour, which acts as a signal to the observer, which then changes its emotional state and behaviour, which then alters the state, and ultimately behaviour, of the actor (Figure 14).

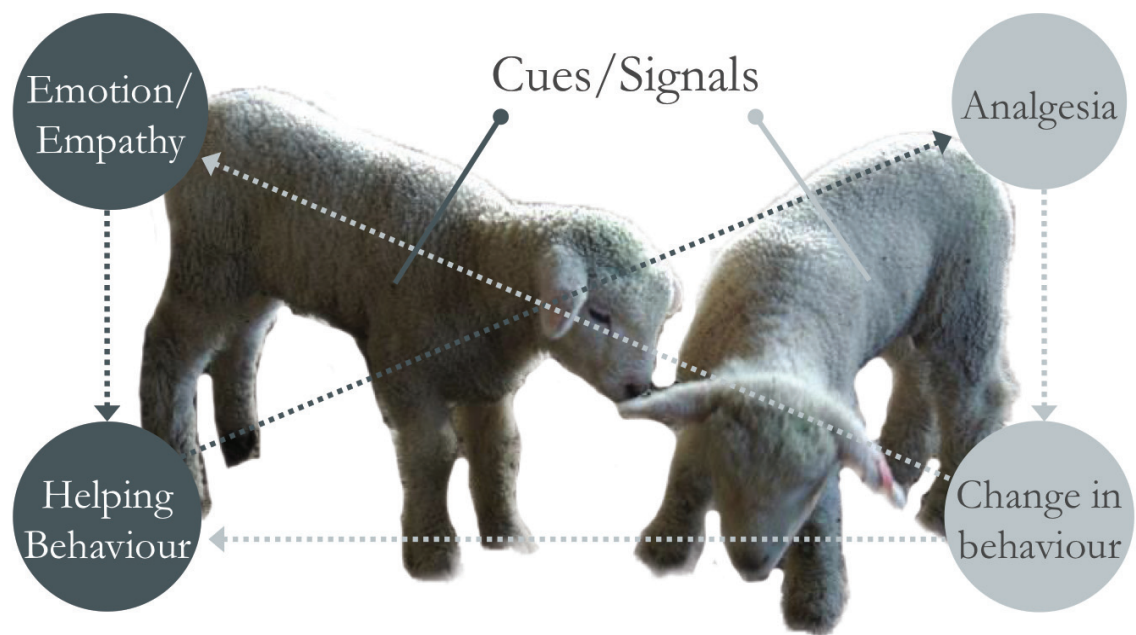


Figure 14: The cycle of interaction between the observer lamb (dark grey circles) and actor lamb (light grey circles). A change in behaviour by the actor (1) may induce a state of empathy in the observer via activation of the same neural networks (2) which drives either helping or another emotion such as fear which drives avoidance behaviour by the observer (3). This change in behaviour by the observer may lead to analgesia in the actor via social buffering through tactile contact and opioid release (4) and a subsequent change in behaviour (5) or alter the actor's behaviour by some other mechanism. Note that both lambs' behaviour may act as a cue or signal to the other lamb as well as heterospecifics.

Table 21: Summary of previous research into socially facilitate behaviour in observer animals. Cagemates refers to partners that are familiar with one another, that is have been reared together, but unrelated. Strangers refers to partners that are unfamiliar with one another, that is raised apart, and unrelated. Table continues on next page.

Paper	Study animal	Relationship with partner	Pain stimulus	Observer behaviour measured	Findings	Conclusions
Church, 1959	Rat	Cagemates	Electric shock	Frequency of bar pressing when bar pressing resulted in food for the observer	The frequency of bar pressing by the observer decreased if this was also associated with a shock to the actor	Showed pro-social behaviour
Rice, 1964	Mice	Cagemates	Electric shock	Frequency of bar pressing when bar pressing resulted in food for the observer	The frequency of bar pressing by the observer decreased if this was also associated with a shock to the actor	Showed pro-social behaviour
Walker et al., 2003	Rat	Mother of actor	Heel prick	Care behaviour in the form of retrieval time, pup-grooming time, nesting time.	Mother showed more care behaviour when offspring was in pain than when it was being handled or no treatment	Showed pro-social behaviour when offspring in pain but not stressed.
Langford et al., 2006	Mice	Cagemates	Injection of irritating substance into abdomen	Writhing (pain behaviour)	Observers writhed when viewing another mouse in pain even when not injected themselves. Magnitude of writhing greater if both injected.	Showed socially facilitated behaviour in the form of mirroring
Edgar et al., 2010	Sheep	Mother of actor	Tail-docking and castration	Care behaviour (specifics unknown only abstract available) and grooming	Mother showed more care behaviour when offspring was in pain than after lamb had been socially isolated or no treatment	Showed pro-social behaviour when offspring in pain but not stressed.

Langford et al., 2010b	Mice	Cagemates or strangers either male or female but always in same-sex pairs	Injection of irritating substance into abdomen	Time spent in close proximity to pained, trapped individual	Only females spent more time in closer proximity to a pained conspecific. Males spent same amount of time with them as elsewhere. Mice approached familiar pained mice more often than unfamiliar	Showed helping/pro-social behaviour and this depends on sex and relationship to actor.
Hild et al., 2011	Sheep	Mother of actor	Tail-docking for female offspring, castration for male offspring	Care behaviour in the form of maternal sniffing, licking, glancing and nursing	Mother showed more care behaviour when offspring was in pain than after lamb had been socially isolated or no treatment	Showed pro-social behaviour when offspring in pain but not stressed.
Colditz et al., 2012	Lamb	Familiar but unrelated lamb also in pain or familiar but unrelated lamb not in pain	Castration	Proportion of time spent engaged in the same behaviour at the same time. Plasma cortisol concentrations.	No increase in pain-related behaviour in the pained partner and no mirroring behaviour in the non-pained partner.	Presence of an unrelated lamb in pain had no effect on behaviour or physiology.
Guesgen et al., in preparation	Lamb	Either twin lambs (Familiar Related), Familiar Unrelated, strangers	Tail-docking by rubber ring	Pain-related behaviours, ear posture, where lamb was looking	All lambs (regardless of relationship) displayed some of the same pain-related behaviours and showed signs they were in a negative emotional state (ears back). Twins mirrored looking at tail behaviour.	Showed socially facilitated behaviour in the form of mirroring. This was affected by relatedness.

Table 22: Summary of previous research into buffering in actor animals due to observer presence or behaviour. Cagemates refers to partners that are familiar with one another, that is have been reared together, but unrelated. Strangers refers to partners that are unfamiliar with one another, that is raised apart, and unrelated. Table continues on next page.

Paper	Study animal	Relationship with partner	Pain stimulus	Behaviour measured	Findings	Conclusions
D'Amato et al., 1998	Mice	Sibling or stranger	Heating pad on tail	Time until tail wagging or lifting, interpreted as a nociceptive response, was observed by actor. Paw licking	Longer latency to respond (less sensitive to pain) when with a sibling mouse. Displayed less paw licking when with a familiar individual than when alone.	Shows social buffering and that social buffering is affected by relationship with partner. Shows social buffering and that social buffering is affected by relationship with partner.
Gioiosa et al., 2009	Mice	Cagemate or stranger	Heat pad on foot	Paw licking	Displayed less paw licking when with a familiar individual than when alone.	Shows social buffering and that social buffering is affected by relationship with partner.
Hild et al., 2010	Lamb	Observer was mother	Heating pad on tail	Time until tail wagging or lifting, interpreted as a nociceptive response, was observed by actor. The distance between the ewe and lamb was measured as well as their synchrony (performing the same activity) by mother.	When ewe and lamb were close and rested together lambs had a longer latency until nociceptive response.	Lambs undergoing a painful test with dam were less sensitive to pain than when alone. Positive mother-young interaction decreases lamb pain.
Langford et al., 2010b	Mice	Cagemate or Stranger	Injection of irritating substance into abdomen	Pain-related behaviour in the form of writhing	Displayed less writhing when proportional to the amount of time the cagemate spent in close proximity (but not the stranger).	Shows social buffering and that social buffering is affected by relationship with partner.

Langford et al., 2011	Mice	Stranger	Injection of irritating substance into abdomen	Pain-related behaviour in the form of writhing	When the pair were separated by a Perspex barrier, the actor showed a greater frequency of writhing. When not separated, the actor showed a reduction in writhing.	The presence of a tangible social threat was associated with social buffering.
Colditz et al., 2012	Lamb	Familiar but unrelated lamb	Castration	Pain-related behaviours e.g. rolling, kicking, stamping, time spent in abnormal postures	No effect of an observer lamb to the frequency of pain-related behaviours displayed.	No evidence for social buffering of pain.
Guesgen et al., 2014	Lamb	Either twin lambs (Familiar Related), Familiar Unrelated, strangers	Tail-docking by rubber ring	Pain-related behaviours e.g. rolling, kicking, stamping, time spent in abnormal postures	Lambs with a twin showed a smaller increase in rolling than other groups	Shows social buffering and that social buffering is affected by relationship with partner.

Effects on observers

Socially Facilitated Behaviour

A change in the behaviour of an observer in the presence of an actor is referred to as socially facilitated behaviour (SFB) (Clayton, 1978). There is evidence for SFB in the context of pain from several non-human mammalian species. For example, observer mice showed writhing (a pain behaviour) when viewing another mouse writhing after injection of an irritating substance into the abdomen, although they themselves had not been injected (Langford et al., 2006). In addition, the magnitude of writhing behaviour of both the actor and observer was greater when both mice were injected simultaneously than when only one mouse of the pair had been injected or when the actor was injected in isolation. This highlights that the interaction between the actor and observer is reciprocal as the behaviour of the observer also altered the behaviour of the actor.

Mice have also been shown to exhibit pro-social behaviour in response to a conspecific in pain. Pro-social behaviour refers to helping behaviour, be it successful or attempted (Langford et al., 2010b). Pro-social behaviour occurred in the form of closer proximity to a pained mouse that was free or towards a trapped mouse in pain (Langford et al., 2010b; Watanabe, 2012). Pro-social behaviour also occurred in the form of decreased frequency of bar pressing to obtain food when this resulted in an electric shock to a conspecific (Church, 1959; Rice, 1964).

Previous work on non-human SFB relating to pain has focused on rodent models, but sheep have also been shown to express SFB. All observer lambs, regardless of the relationship between the actor and observer, spent more time looking at the actor, head-butted it more often and showed an increase in active behaviour compared to when the actor was not in pain (Chapter 4). All observer lambs also spent more time with their ears backward, indicative of a negative emotional state, when in the presence of the pained actor (Chapter 4). Like previous studies of SFB associated with pain (e.g. Langford et al., 2006) We found that related observer lambs were also mimicking or engaging in some of the same pain-related behaviours as the actor lamb including lip curling, head shaking, jumping, stamping and tail wagging (Chapter 4).

The expression of socially facilitated behaviour depends upon the relationship between the observer and actor. Because pain represents an aversive or potentially threatening stimulus, we would expect parents to attend to, and subsequently alter their behaviour in response to pain behaviours of their offspring (Penner et al., 2008). Three studies of non-human mammals highlight the maternal response to the pain of offspring. Both rats (Walker et al., 2003) and sheep (Edgar et al., 2010; Hild et al., 2011) showed an increase in grooming and care behaviours after their pup or lamb had experienced a painful event (heel-prick in the case of rats, tail docking or castration in the case of lambs), but not when they had experienced a stressful one (handling or social isolation). These studies support the idea that the mother is recognizing and responding to aspects of her offspring's behaviour that

are indicative of pain (Edgar et al., 2010; Hild et al., 2011; Walker et al., 2003).

Few non-human studies have investigated the effect of viewing a conspecific in pain on a related observer animal that isn't a parent. The presence of an unrelated lamb in pain had no effect on the behaviour or physiology of an observer lamb who was also experiencing pain (Colditz et al., 2012). In my studies, related lambs looked at their tail more after actor docking than other groups and tended to be rated as more comforting and consoling on a qualitative assessment scale (Chapter 4). This suggests that, like in rodents, the relationship between the observer and actor determines the effect of an actor in pain on an observer sheep. Familiarity between actor and observer is also important, with mice approaching familiar actors more than unfamiliar ones (Langford et al., 2010b).

Pain and social learning

Information from signals and cues can alter behaviour of the observer immediately. In addition, if the information is retained and used subsequently when the actor is not present this can be referred to as social learning (Nicol, 1995). Here, learning is defined as a change in behaviour as a result of experience (Krause and Ruxton, 2002).

Social learning about pain can be advantageous as it allows animals to avoid potentially harmful stimuli in the environment without having to experience the noxious stimulus themselves (Kavaliers et al., 2005). It is also advantageous for an animal to be able to learn about particular details of the environment, as opposed to solely possessing a generalized innate pain avoidance strategy, as features of the environment may change. For example, a new noxious plant or predator may be introduced. Social learning therefore allows the animal to alter its behaviour in response to environmental changes (Griffin, 2004).

A variety of mammals have demonstrated the ability to learn to avoid potentially painful stimuli through social means (Griffin, 2004; Griffin et al., 2010; Griffin and Evans, 2003; Lindeyer and Reader, 2010; Mateo, 1996; Shier and Owings, 2007). In many cases, this takes only one exposure to the conspecific actor (Griffin, 2004). Predators, particular locations or environmental features all represent stimuli that animals can learn to avoid in order to avoid potential pain (Griffin, 2004). Pain-related behaviour of a conspecific may act as a signal or cue to draw attention to such stimuli, although this has not been specifically tested. However, Rhesus monkeys who had learned to avoid a predator by viewing a conspecific actor in pain, were then still able to teach other naïve monkeys to generate a similar appropriate response without the presence of a pained actor (Cook et al., 1985), suggesting that pain-related behaviour facilitates social learning.

There are three, non-mutually exclusive explanations for how learning may occur. Firstly, the salience of the stimulus may be increased by the presence of the signal/cue sender (actor). The observer's attention is then drawn to previously ignored features of the environment (Nicol, 1995). This is more likely for "neutral" features such as particular locations or environmental features, as opposed to predators.

Alternatively, imitation theory infers that the observer copies the motor patterns of the actor experiencing pain and, through doing so, learns (Nicol, 1995). Finally, a form of operant learning may occur whereby the observer associates a previously neutral stimulus (for example a hole in the ground) with the negative emotional state evoked by the viewing the behaviour of a conspecific for example limping associated with a broken limb (Griffin and Evans, 2003; Panksepp and Panksepp, 2013). Here, limping may both have the function of facilitating healing but, perhaps inadvertently, it also has a social function. The experience of pain can therefore serve as a stimulus for learning, both in the individual experiencing it (see section “Avoidance function of pain”), and vicariously in an observer through viewing pain-related behaviour in an actor.

Effects on actors

Social Buffering

The presence of an observer can alter the emotional state of an actor as indicated by its behaviour. For example, the presence of a conspecific may have an analgesic or calming effect on the actor (Langford et al., 2010b). Such “social buffering” has been demonstrated in a number of mammalian species (Kikusui et al., 2006) including humans (Thorsteinsson et al., 1998), non-human primates (Coe et al., 1982; Levine et al., 1997; Mendoza et al., 1978), guinea pigs (Hennessy et al., 2000), rats (Davitz and Mason, 1955; Kiyokawa et al., 2012; Morrison and Hill, 1967) and sheep (Ligout and Porter, 2004; Porter et al., 1995). Social buffering of pain states is of particular interest, as it may allow animals to recover, in terms of feeling less pain, more quickly from an aversive experience (Kikusui et al., 2006). In addition, social buffering, in terms of feeling less pain, may be ultimately beneficial as it reduces the behavioural signs of pain of a conspecific, reducing the likelihood of possible predation (Hamilton, 1964).

As stated previously, the genetic-relatedness between individuals influences the likelihood of interaction, as well as the type of interaction. Parents represent the closest genetic relatives and so parents are a likely source of comfort for a pained individual. While there are numerous studies looking at the effect of parental buffering of pain in humans (Akcan et al., 2009; Chambers et al., 2002; Chambers et al., 2009; Dodd, 2005; Sajedi et al., 2007), there are very few in non-human mammals. Lambs that were in the presence of their dam when undergoing a painful test were less sensitive to pain than lambs experiencing pain on their own, suggesting that positive mother-young interaction decreases lamb pain sensitivity (Hild et al., 2010). Such interaction may be in the form of grooming or care behaviour (Hild et al., 2011; Walker et al., 2003).

Buffering may also occur between other related conspecifics. Mice were less sensitive to thermal pain when tested with a familiar, related or at least related individual compared to when they were with an unfamiliar, unrelated test-mate (D’Amato, 1998). Mice also displayed fewer pain-related behaviours after being injected with formalin or acetic acid in

the presence of a familiar individual than when alone or with an unfamiliar mouse (Gioiosa et al., 2009; Langford et al., 2010b). There are mixed findings regarding the effects of social context on pain expression in sheep. The presence of a familiar, but unrelated, observer lamb also in pain had no analgesic effect on physiological and behavioural indicators of pain in castrated lambs (Colditz et al., 2012). However, the presence of a twin reduced pain expression after tail docking in an actor lamb (Chapter 3.2).

Unfamiliar individuals are unlikely to engage pro-socially with one another, as it confers little to no fitness advantage. Therefore social buffering is unlikely to occur when the actor and observer are unfamiliar. For example, no analgesic effect was found when a pained male mouse was placed in the presence of an unfamiliar, male mouse who was separated from him by a Perspex barrier (Langford et al., 2011). In contrast, a hyperalgesic effect was found. However, if the mice in this study were not separated by the barrier, analgesia did occur through stress induced analgesia (Langford et al., 2011). This is because unfamiliar individuals, including predators, may represent a threat. A reduction in pain behaviour may therefore be advantageous as responding to a noxious stimulus might compromise effective defense or escape behaviour. In addition, displaying very active forms of pain-related behaviour may further draw unwanted attention to the individual in pain.

Mechanisms underlying Socially Facilitated Behaviour

Once attention has been drawn to an actor, pain behaviour can generate an emotional state in the observer which leads to an appropriate action. As discussed in the introduction, the neural systems in the brain generate emotion or affective states that drive future behaviour (Panksepp, 2011; Panksepp and Panksepp, 2013). When the emotional states of the actor and observer match, we describe this state as an empathic one.

The term ‘empathy’ from a human-focused standpoint is often used interchangeably with the term ‘theory of mind’ or ‘mentalizing’. This refers to the ability to attribute mental states such as desires, beliefs and intentions to another (Singer, 2006). This restricts empathy to a purely human capability (Baron-Cohen et al., 1985; Perner et al., 1987) and can only be measured through verbal report such as the Empathic Concern Scale (Davis, 1980) or the Balanced Emotional Empathy Scale (Mehrabian and Epstein, 1972).

In order to move away from a human-only-focused definition of empathy, it is useful to think of this capability as the shared emotional state between two individuals, which leads to the generation of an appropriate action (Preston and de Waal, 2002). This fits well with Panksepp’s definition of emotion, as being a state that precedes and drives behaviour through particular neural systems. Also, in order to accommodate other non-human species, it is useful to adopt a “bottom-up” approach (Panksepp and Panksepp, 2013). This also allows us to postulate how empathy may have evolved, by looking at the different complexity levels of empathy. At the most primal or basic level, empathy represents an ability to share an emotional state with an individual and can cover terms such as

‘emotional contagion’ and ‘affect matching’ (Figure 15) (Panksepp and Panksepp, 2013).

Underlying this level of empathy are the neural networks that are activated both when an individual experiences an emotion, and when they view that emotion in a conspecific (Baird et al., 2011; Casile et al., 2011; Decety, 2010; Decety and Meyer, 2008; Ferrari et al., 2005). In other words, the same brain areas are activated in both the actor and observer. Evidence for this neural network has thus far only been reported in humans and non-human primates, however the generation of empathic or socially facilitated behaviour suggests the presence of some form of this system in other mammals (D’Amato, 1998; Gioiosa et al., 2009; Hild et al., 2010; Hild et al., 2011; Langford et al., 2010b; Walker et al., 2003).

The next level of empathic complexity describes empathic habits, or, the inclusion of memory and learning processes (Panksepp and Panksepp, 2013). This extends beyond the immediate behavioural response of an observer and includes learned behaviours that result from the ‘rewarding’ or ‘punishing’ effects of viewing an actor. This level differs very little from the primal level, except that the response of the observer is separated from the behaviour of the actor by time (Panksepp, 2011). Neural structures associated with this level of empathy include areas of the basal ganglia and upper limbic systems. Evidence for this level of empathy are the numerous studies on social learning described in the previous section “Pain and Social Learning” (Griffin, 2004; Griffin et al., 2010; Griffin and Evans, 2003; Lindeyer and Reader, 2010; Mateo, 1996; Shier and Owings, 2007).

The third level requires the animal to be cognitively evaluating the situation and therefore promoting conscious decision-making and executive control of behaviour (Edgar et al., 2012; Panksepp and Panksepp, 2013). This involves largely cerebral brain regions as well as limbic regions (Panksepp and Panksepp, 2013). This level describes the level of empathy experienced by humans (Watt, 2007) and is best self-reported. There is some evidence, from a non-verbal version of a Theory of Mind test, that chimpanzees are also able to experience this level of empathy (Call and Tomasello, 1999). However, it is thus far unknown whether other mammals are also able to experience this level of empathy.

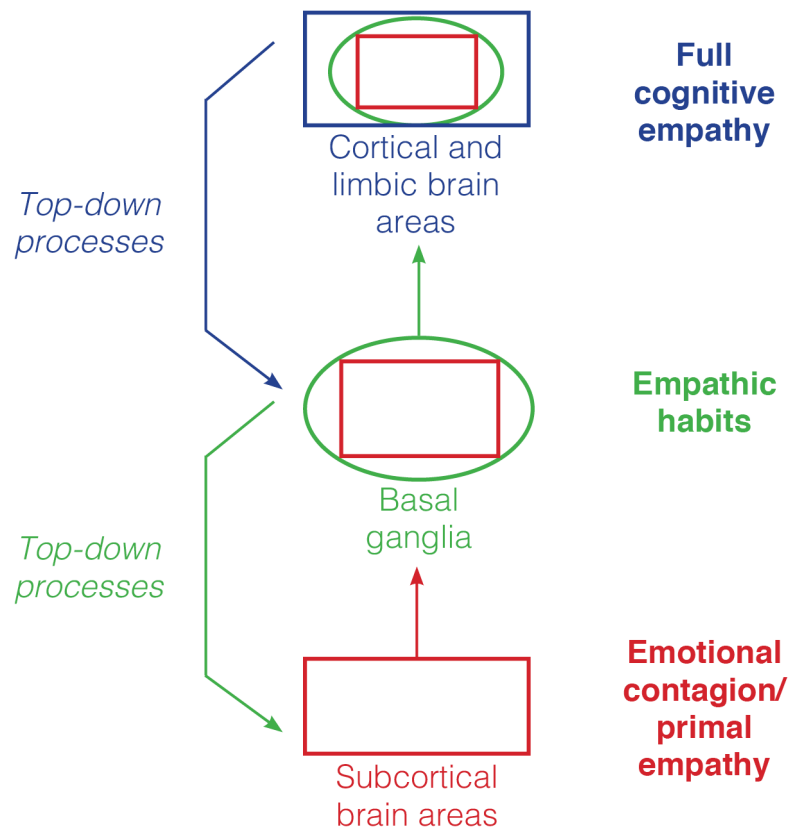


Figure 15: The nested mind-brain hierarchies of empathy. Boxes and ellipses (middle) represent the brain areas associated with the different levels of empathic processing (right). Note how these structures become nested within one another as the complexity of brain organization increases through a “bottom-up” (arrows, middle) process. Arrows (left) demonstrate that “top-down” regulation or control can also occur. Figure based on Panksepp & Panksepp (2013).

It has been suggested that animals can experience emotions, such as empathy, without actually “feeling” them (Edgar et al., 2012). For example, proponents of this ideology say it is possible for an animal to display socially facilitated behaviours or pro-social behaviours that appear to be due to distress, but the animal itself is not actually ‘distressed’. Partly, this comes from a trepidation to attribute anthropomorphic terminology to other mammals. However a new approach to behavioural and neuroscience research is to see the animal not as separate physiological, emotional and behavioural parts but as a whole whereby the outward displays of body posture and movement reflect the emotional state of the animal (Wemelsfelder et al., 2001). In addition, all mammals share neural emotional-action systems where behaviour is guided or initiated by affective states (Panksepp and Panksepp, 2013).

The alternative explanation for SFB is that suggested by Imitation Theory. This implies a more simple reproduction or copying of behaviour that is reflexive and occurs without any shared emotional state underlying it (Zentall, 2001). The simplest forms of imitation include SFBs that occur in unison such as flocking or herding, or feeding. These do not require animals to share a mental state and benefit the individual by allowing it to avoid appearing conspicuous within a group. Such SFB occurs by the automatic activation of neurons that fire both when viewing an action and executing it (Hecht, 2012). This alternative therefore comes back to the problem of how emotion is defined. If we define emotional systems as Panksepp does, as being a state that precedes and drives behaviour through particular neural systems, then imitation theory is no different to primary level empathy under the Panksepp model.

Mechanisms underlying Socially Facilitated Behaviour

Social buffering may be facilitated by systems which reward the animal when it is in close contact with another group member (Kikusui et al., 2006). Visual (de Costa et al., 2004; de Costa et al., 2000; Morton and Johnson, 1991), tactile (Latane, 1969; Nelson and Panksepp, 1998; Wilson, 2001), auditory (Rukstalis and French, 2005; Snowdon and Cleveland, 1980) or olfactory (Agren et al., 1997; Guiraudie et al., 2003; Kikusui et al., 2001; Kiyokawa et al., 2004; Sheppard and Mills, 2003) cues or signals may all facilitate this affiliation, with oxytocin being the hormone underlying social affiliation in mammalian species (Insel, 1997; Winslow and Insel, 2002; Young, 2002; Young and Wang, 2004). The mechanism underlying social buffering may be an increase in opioid release through tactile contact (Kikusui et al., 2006). There is evidence in mice that the administration of an opioid antagonist results in a decrease in social contact between mice and ultimately a decrease in analgesia (D'Amato and Pavone, 1996). The mechanisms underlying social buffering through other cues remains unclear.

Other factors influencing the social function of pain behaviour

We have described throughout this review how and why the factors of familiarity and relatedness influence the expression of pain by actors, and the behavioural response of observers. At least two other factors may influence either socially facilitated behaviour of observers or social buffering of pain in actors, these are sex and previous experience of pain.

Altering one's behaviour based on the sex of a social partner is important as that partner could either represent a potential mating opportunity or a social threat (Halliday, 1981). There is evidence that an observer mouse's or an observer lamb's response to conspecific pain differs according to sex. Only female mice spent their time in closer proximity to a trapped, pained mouse, with males spending equal time with a free, unaffected cagemate and a trapped one in pain (Langford et al., 2010b). Female observer lambs were perceived as more active and alert, according to a qualitative assessment scale, when the actor was in pain compared to when it was not (Chapter 4). However, male lambs engage in more active and aggressive play than do females (Sachs and Harris, 1978), meaning they may have been more active than females before the pain stimulus was applied. Because of the high level of activity that male observers displayed before the pain stimulus was applied, human observers may not have detected a noticeable change in behavioural expression in male lambs. Thus sex differences in play behaviour may have obscured any change in behaviour when the actor was in pain (Lynch et al., 1992). After the pain stimulus, both males and females were more likely to be paying attention to their test-mate in pain, and as a result displayed equally active behaviour.

There is also evidence that the sex of actor and observer lambs has some minor effects on actor pain behaviour. For example, observer sex affected rump wagging, a pain behaviour, after docking, with female lambs showing less wagging in the presence of a male observer than with a female observer (Chapter 3.2). Female actor lambs also spent more time looking at a male observer over the whole testing time (Chapter 3.2). However, as there were no consistent effects on multiple behaviours, the relevance of these sex relationships for the expression of behaviour is currently unclear.

Previous experience observing pain may alter the expression of pain by an actor. For example actor lambs showed lower overall activity and a less pronounced response to docking pain when they had seen a conspecific undergo the same procedure previously. This may be due to either the greater familiarity with the test environment, for example being less fearful or stressed by it, or the altered interaction between the lambs, leading to more effective social buffering from their partner (Chapter 3.2).

It is unlikely however that previous experience of pain is needed to generate SFB by an observer, because the neural mechanisms underlying at least the primal level of empathy are present in all mammals (Panksepp, 2011). We know from studies of people with congenital insensitivity to pain that previous experience of a painful event is not necessary to elicit an empathic response. Patients still show activation in the brain areas associated with a painful experience when they view a painful stimulus being applied to an actor's body (Borsook and Becerra, 2009). Experiencing a painful event is also not necessary for social avoidance learning to occur. For example Rhesus monkeys who had learned to avoid a predator by viewing a conspecific actor in pain, were then still able to teach other naïve monkeys to generate a similar appropriate response without the presence of a pained actor (Cook et al., 1985), suggesting that pain-related behaviour facilitates social learning.

Conclusion

In this review we have presented evidence supporting a social function of pain behaviour, demonstrating how, in a number of mammalian species, pain behaviour can result in a change in emotional state and behaviour of an observer and how this change may lead to the alleviation of pain in an actor via social buffering. However more work needs to be done in this area. There are several questions to address:

- Why would animals have facial expressions for pain or show ear posture changes if not for communication? Is there an alternative explanation?
- Is there an effect of social context on facial expression of pain?
- How would the addition of a third party into the actor-observer dyad affect social buffering or the experience of empathy and subsequent SFB?
- What sort of evidence would we need to provide to be confident that an animal is experiencing empathy at the second or third stage?
- How do we disentangle the underlying mechanisms from the outward behaviour?
- Does the severity of painful experience in the past alter empathy and SFB in observers?
- Do solitary animals' and social animals' expressions of pain differ? And if so, how?
- How does a social animal's pain expression when they are alone differ from when they are with other group members?

The social function of pain behaviour is an interesting and worthwhile avenue of investigation and we encourage further discussion on the topic, as well as encouraging others to generate data relating to the social aspects of pain behaviour.

General discussion



In the series of experiments presented in this thesis I have developed and utilized some novel methodologies to assess pain-related behaviour in lambs. This section discusses these techniques, their applications for research or on farm, and how they can be investigated further or improved in the future. It also draws together the findings of the experimental chapters of the thesis and assesses them in light of the social function of pain behaviour, which was presented in Chapter 5.

General discussion

6

Discussion of methodologies and their limitations

A key area of research for this thesis has been the development and/or testing of novel assessment methods for pain in lambs. These include: ear postures and facial expression. In addition, I wanted to apply a Qualitative Behavioural Assessment methodology in a novel way for assessing socially facilitated behaviour (SFB) and empathy in lambs viewing a conspecific in pain. This brief section discusses these techniques, their applications on farm or for research, and how they can be investigated or improved in the future.

Ear Posture

Ear posture, or the frequency of postural changes, have been shown to reflect various emotional states of animals (Fox, 1971; Williams, 2002). In adult sheep, the ‘forward’ ear posture has been associated with negative experiences whereas the ‘plane’ posture has been associated with positive ones (Boissy et al., 2011; Reefmann et al., 2009a; Reefmann et al., 2009b; Stubsjoen et al., 2009; Veissier et al., 2009). I investigated whether ear postures related to the experience of pain in lambs and, if so, whether they were influenced by factors such as social context (Chapter 2.1).

I found that pain induced by tail-docking was associated with an increase in the proportion of time spent with Ears Backward and a decrease in the proportion of time spent with Ears Plane (Chapter 2.1). There was also a significant increase in the number of changes between ear postures from pre- to post-docking (Chapter 2.1). Previous studies interpreted the forward ear posture as indicative of negative emotion, specifically elicited by separation from other group members (Reefmann et al., 2009a; Reefmann et al., 2009b; Stubsjoen et al., 2009). It is likely that separation from the flock will elicit increased alertness or arousal as the sheep tries to reunite with the group, whereas the backward posture is associated with the negative experience of pain and the uncontrollability of that experience (Boissy et al., 2011; Coulon et al., 2011; Moe et al., 2006). Pain, by definition, is a negative experience (Molony and Kent, 1997). Changes in ear posture occurred alongside validated behavioural changes that indicate the lamb is in pain, therefore we can be confident that these ear posture changes represent a negative emotion in lambs.

Because ear postures are highly visible, they allow for a non-invasive indicator of emotion in lambs. This may make ear posture a useful technique for quick, on-farm welfare assessment. However, ear postures are limited in the level of detail they provide. In essence, there are only four potential ear postures a lamb or sheep can display (forward, backward, plane or asymmetrical). Ear postures only represent basic emotional valence (i.e. positive, negative, or neutral emotional states) and the same ear posture may be displayed in a variety of situations. They are also strongly influenced by multiple factors as illustrated by the opposite results when sheep were socially separated and can change suddenly in response to social context or other environmental events. It is therefore difficult to assess particular emotional or mental states from ear posture alone. A limitation to the study design is that I did not have a control group where neither lamb was docked (Chapter 2.1). We initially tried to allocate lambs into such a group, however only eight lambs were available and this would not have been sufficient for any statistical analysis. Therefore I was unable to say how ear postures may have changed over time due to factors other than pain.

I have validated ear postures for pain to some extent by relating them to previously-validated pain-related behaviours and by using them as part of the Lamb Grimace Scale (LGS)(Chapter 2.2). Human observers rated ear postures consistently using the LGS in Experiment II (average consistency = 0.86) and scores for this feature increased significantly after docking. However, there were no significant quantitative changes in ear posture but this may have been due to difficulties in measurement (described in Chapter 2.2).

In future, it may be useful to also correlate ear postures with physiological measures of pain. I used tail-docking to induce pain in lambs, but it might also be interesting to see the response of lambs and sheep to other painful experiences, including chronic conditions like foot rot or other forms of docking such as the hot iron. Different sheep breeds could also be used to see if pain is expressed differently by different breeds.

Facial Expression

Ear posture may be part of a wider facial expression for pain in lambs. Facial expressions are routinely used to assess pain in humans, particularly non-verbal humans. Recently, there has been an interest in developing coding systems for facial grimacing in non-human animals, such as rodents and rabbits (Keating et al., 2012; Langford et al., 2010; Sotocinal et al., 2011). I aimed to identify whether lambs produce noticeable changes in facial expressions when experiencing pain and then to develop a 'Grimace Scale' that incorporates these expressions (Chapter 2.2).

The Lamb Grimace Scale (LGS) was devised in consultation with scientists experienced in assessing facial expression in other species by comparing images of docked lambs before (no pain) and after (pain) docking. The LGS consists of five facial action units: Orbital Tightening, Mouth Features, Nose Features, Cheek Flattening and Ear Posture. The LGS was tested across two experiments by having groups of five observers use the scale to evaluate the presence of pain in lambs. In Experiment II each facial action unit was also quantified using image measurement software.

The LGS may be a valuable method for assessing pain in lambs, however more work needs to be done to determine its validity and usefulness. There was little agreement between experiments as to which features observers scored differently after docking and the changes in observer scores for experiment II did not match up with the features that changed quantitatively. Furthermore, observers scored changes in control lambs in Experiment I although these lambs were not in pain. This may indicate one of two things, either that there is no consistently recognized facial expression for pain in lambs, or that limitations of the methodology meant I did not see more consistency between experiments. It is more likely that limitations of the methodology meant I did not see more consistency. It has already been validated that lambs display other body behaviours related to facial grimacing such as lip curling and neck arching (Molony and Kent, 1997). The muscles used to express other pain-related behaviours such as lip curling may also be associated with muscles that may be used for particular facial expressions. Therefore grimacing and other pain related behaviours may occur concurrently. In addition, other research institutes have identified a similar grimace scale in adult sheep (unpublished data, Krista McLennan, personal communication). Future studies should use greater lamb numbers, making sure lambs are filmed unrestrained, develop a system whereby the camera angle is maintained throughout filming, and train all human observers well before testing.

Like ear posture, facial expression is a fairly non-invasive technique to assess pain, and it has potential welfare implications, in sheep once the scale is properly validated. There is greater subtlety of facial expression compared to ear postures, therefore the LGS could potentially be used to indicate the severity of pain, just as human grimace scales do. However, unlike ear postures, learning to code facial expression takes more time and practice. I have identified inconsistencies among observers, which may be due to differences in training before LGS scoring.

Qualitative Behavioural Assessment

Another aim in this thesis was to investigate how pain expression affects the emotional expression and behaviour of conspecifics (Chapter 4). This was done using a novel application of the Qualitative Behavioural Assessment (QBA) technique.

QBA is a 'whole animal' approach which integrates many features of the animal's behaviour such as body posture, movement and context (Wemelsfelder et al., 2001). QBA views the animal as an agent for their own behaviour (Wemelsfelder, 1997). As such the

animal's behaviour characterizes its state and experience, which may change over time (Wemelsfelder et al., 2001). QBA has mostly been developed and tested using a Free Choice Profiling (FCP) method; a creative process where human observers generate their own terms to describe an animal's behavioural expression and use these terms to score animals in various situations.

Previous studies have used QBA to assess factors that may significantly affect the welfare of an animal, such as housing, novel environments or transport (Cockram et al., 2012; Minero et al., 2009; Napolitano et al., 2008; Napolitano et al., 2012; Phythian et al., 2013; Rousing and Wemelsfelder, 2006; Rutherford et al., 2012; Stockman et al., 2011; Stockman et al., 2012; Walker et al., 2010; Wemelsfelder et al., 2012; Wemelsfelder et al., 2000; Wickham et al., 2012). I found QBA useful for our purposes, because there were no prescribed descriptors or ethograms of behaviour to describe how an observer lamb would react to viewing a conspecific in pain. The FCP method was therefore flexible and accommodating for the variety of behaviours I encountered when viewing observers' reactions.

The terms generated by human observers were interesting in and of themselves, especially as observers were unaware of the allocation of lambs to different treatment groups and were not told which video clip related to the pre- or post-docking period. Observers were none-the-less able to distinguish between the behavioural expression of lambs who were viewing a conspecific in pain and those whose test-partner was not in pain. Having said this, it may also be that their judgements were biased by seeing the actor in the same clip, although they were told not to focus on that particular lamb. An earlier paper using QBA identified how the context of the video influenced observers' scores of behaviour (Wemelsfelder, 2000). It would be interesting to conduct the study again but blind participants to the context by cropping videos where possible to only include the observer lamb.

This technique is potentially the most time-consuming of the techniques described in this thesis. Having said that, it also yields some of the most interesting data. Human observers described observer lambs' behaviour differently according to their sex and treatment group. In addition, while QBA is creative, it is also valid and reliable and compliments traditional behavioural and physiological methods well (Wemelsfelder et al., 2001). I used video recordings of our animals, however QBA can also be used "live" on-farm (Wemelsfelder et al., 2000), making it an informative as well as non-invasive methodology.

As already discussed in Chapter 4, the selection of clips may have inadvertently biased the terms used to describe particular groups of lambs, specifically twins. Using QBA "live" could potentially remedy this. However then comes the challenge of trying to blind observers to the study design. Again, the inclusion of a control group in this experiment would have been useful to see how observer lamb behaviour changes over time without the stimulus of actor docking.

Conclusions

I have not only utilized, but also developed, some novel methodologies alongside a traditional pain-related behaviour ethogram. I have demonstrated how ear postures relate to the negative experience of pain, that lambs potentially show grimacing in response to pain, and used Qualitative Behavioural Assessment to investigate lamb behaviour and emotional expression. All of these methods show promise for development into useful welfare indicators.

Interpreting the results of experimental studies

The previous section of this chapter has discussed one of the primary foci of this thesis, namely finding novel ways to measure pain-related and social behaviour in lambs.

As part of this aim, and in order to know which factors may affect pain expression, it was important to include the experimental study outlined in Chapter 3.1. This chapter investigated the effects of age and sex on baseline pain sensitivity, rather than on pain induced by injury (e.g. docking) as in other chapters. This study was conducted early on in the PhD and highlighted the need to include sex as a factor when considering pain expression through behaviour. In accordance with this, sex of the observer was found to have an effect on the behaviour of female actor lambs (Chapter 3.2). Female actor lambs showed less rump wagging, a validated pain behaviour, when the test-mate was male, and females spent more time looking at a male observer over both periods in round 1. It is suggested that the presence of a male is stressful or distracting to the female, however these conclusions require further investigation. Sex of the observer also had an effect on how the observer was perceived by human observers using QBA (Chapter 4). Female observer lambs were rated as more alert and active in the post-docking period compared to the pre-docking period. Again, this may suggest greater vigilance by females to potential threats, like the pain behaviour of the actor lamb.

However the main goal of this thesis was to look at pain-related behaviours from an evolutionary perspective. Three key functions of pain behaviour have been proposed: avoidance, healing, and a social communicative function. In terms of the behaviours recorded in this thesis, it appears that postural body behaviours, such as abnormal lying or lateral lying, may function mostly as behaviours that promote healing. More active body behaviours, such as kicking, rolling, or jumping, may function either to avoid the painful stimulus or as a form of communication. It is more likely that active behaviours draw the attention of observers as opposed to more static behaviours such as abnormal lying. Interestingly, headbutting has previously been associated with the experience of pain by lambs (Molony and Kent, 1997) but, as noted in Chapter 3.2, it also reflects aggression so may serve a communicative function.

I have demonstrated that lambs hold their ears back when in pain. It is unlikely that they are holding them in this way to hear what is happening behind them, as the auricles of the ears are facing downwards. I also see no apparent healing or avoidance function for ear postures, so it is likely that ear postures are mainly an indicator of emotional state in lambs and therefore serve a potential social/communicative function. Similarly, facial expressions are also mainly communicative but may also function to protect the eyes and thus avoid further pain.

A particular focus of the thesis was on the social function of pain behaviour. Chapters 3.2 and 4 in particular focussed on providing evidence for a social/communication function of pain-related behaviour, although social context was also investigated as part of chapter 2.1. This was done either by evaluating how the expression of pain was influenced by the social context or by investigating the effect of viewing pain-related behaviour on an observer lamb. In particular, twin (FR) lambs consistently behaved differently across all studies compared to unrelated or unfamiliar lambs. For example, only twin lambs showed a significant increase in the time spent with ears asymmetrical as a result of docking. Twin actor lambs also rolled less after docking than other groups and headbutted less over both periods compared to unfamiliar lambs. Twin observers mirrored the behaviour of the actor by looking at their own tail more after the actor was docked and they were also rated as more comforting and consoling by human observers. From these results, it appears that the quality of interaction between twin lambs is different from that of other groups.

These findings are consistent with the prediction of evolutionary theories of social behaviour, that the type of social interaction will depend on the relationship between individuals. It is advantageous for individuals to engage in behaviours that promote the survival of those most closely related to them, as these individuals carry 25%-50% of the same genes (Hamilton, 1964). The display of pain behaviour by the actor lamb may elicit a shared emotional state, i.e. empathy in the observer. Evidence to support this idea includes the mirroring behaviour displayed by twin observers, and the increase in the ears backward posture, which has been linked to a negative emotional state in lambs (Chapter 2.1). In twins, this empathic state may lead to pro-social comforting behaviour by the observer in the form of greater positive tactile contact. In turn, this leads to social buffering of pain in the actor, as seen in the smaller increase in rolling behaviour. Such an interaction may be advantageous for both the actor and observer. The actor may experience less pain and subsequently show a less intense pain behaviour response, which is then less distressing to the observer as well as drawing less unwanted attention to the pair.

However, previous studies investigating the effect of social context on pain behaviour in mice found that contact with unfamiliar mice can also produce an analgesic effect (Langford et al., 2011). It is likely that in this case, the stress induced by the potentially threatening unfamiliar individual produced an analgesic effect.

It therefore appears that there are two key avenues that may produce differences in pain perception and pain-related behaviour. One is where a negative shared emotional state between the actor (pain) and observer leads to comforting behaviour and subsequent social buffering. The other, is a state of fear, stress or threat in the actor induced by the presence of an unfamiliar observer, causing a type of stress-induced social buffering.

In the second scenario, it is interesting to consider whether the observer also needs to be in distress, i.e. experiencing empathy, and displaying SFB for the social buffering of the actor's pain to occur. Both situations may be characterized by social buffering of the actor's pain state and/or socially facilitated behaviour by the observer, but whether buffering occurs as a result of comfort or fear is difficult to ascertain. In addition, observation of an actor's pain behaviour can produce different types of SFB, for example pro-social comforting or aggressive threat-induced responses (Langford et al., 2006; Langford et al., 2010). This depends primarily on the relationship between the two and other factors such as sex (Watanabe, 2012) as demonstrated in Chapter 4.

A key question that still remains then, is how to disentangle the potential underlying mechanisms from the behaviour that we see, whether it be ear-related, body behaviours, or facial expressions. It seems that the multi-layered definition of empathy provided in Chapter 5 may be so inclusive that any behaviour by an animal could be explained under this model. An answer may lie in investigating pain-related behaviours such as ear posture or facial expression, once properly validated or to use physiological measures such as cortisol, where an increase may signal threat and a decrease may indicate comfort. As discussed previously, these behaviours appear to have no apparent healing or pain avoidance function and may therefore be a more sensitive measure for investigating the social function of pain. It may be interesting to explore differences in facial expression in the actor depending on their conspecific partner, in a similar study design to what I used in this thesis.

Final thoughts and future directions

When discussing the potential communicative function of pain-related behaviour, I have primarily focused on the interaction between conspecifics. However, the signals or cues animals are giving are also accessible to us. Therefore, behaviour is an important indicator for understanding pain and subsequent welfare implications for a variety of animals. Indeed, a number of behavioural methods including looking at facial expression and qualitative assessments of whole-animal behaviour have been adopted to do just this (Graham et al., 2002; Grant, 2004; Keating et al., 2012; Leach et al., 2012; Lester et al., 1996; Napolitano et al., 2012; Ong et al., 1997; Rousing and Wemelsfelder, 2006; Rutherford et al., 2012; Stockman et al., 2011; Thornton and Waterman-Pearson, 2002, Chapter 2.2, Chapter 4). It is important to recognize, however, that pain behaviour can be influenced by a variety of intra- and inter-animal and environmental factors. These must be taken into account, and their effects further investigated, if we are to use behavioural assessment effectively to understand the welfare implications of pain.

If pain has a role in social communication, we can learn much by studying the effects of pain on the behaviour of solitary animals, particularly in species closely related to social species (for example comparing social lions with solitary lions). Of course, pain behaviours may still serve avoidance or healing functions in such species, but if those animals aren't seeking aid from conspecifics, will they still display the same intensity of observable pain behaviour? As well as differences between social and solitary species, it would be interesting to compare individual behaviour when an animal is in a solitary or social environment. For example humans who are alone are more inclined to display facial expressions of pain than when in the presence of strangers (Kleck et al., 1976).

Different behaviours may also be more or less relevant for different animal species. Humans, for example, pay a lot of attention to faces (Craig, 2009; Williams, 2002). They are highly visible and we gain a large amount of information from reading them (Craig, 2009; Williams, 2002). However, in other animals, body postures or more overt cues may be more applicable due to the proximity of animals to one another or the neural capacity to read and understand certain behavioural expressions (Kendrick, 2006; Kendrick et al., 1995; Kendrick et al., 1996; Kendrick et al., 2007; Kendrick et al., 2001).

The social function of pain behaviour is an interesting and worthwhile avenue of investigation, however more work needs to be done in this area. As highlighted in the literature review paper there are several questions to address:

- Why would animals have facial expressions for pain or show ear posture changes if not for communication? Is there an alternative explanation?
- Is there an effect of social context on facial expression of pain?
- How would the addition of a third party into the actor-observer dyad affect social buffering or the experience of empathy and subsequent SFB?
- What sort of evidence would we need to provide to be confident that an animal is experiencing empathy at the second or third stage?
- How do we disentangle the underlying mechanisms from the outward behaviour?
- Does the severity of painful experience in the past alter empathy and SFB in observers?
- Do solitary animals' and social animals' expressions of pain differ? And if so, how?
- How does a social animal's pain expression when they are alone differ from when they are with other group members?

Conclusion

While the findings presented in the experimental chapters of this thesis contributed a great deal to the understanding of pain-related behaviour from a social/communicative perspective, they also brought to light many new questions which require exploration. Further investigation into the social and communicative functions of pain will better our understanding and potentially our ability to recognize and alleviate pain in other mammals.

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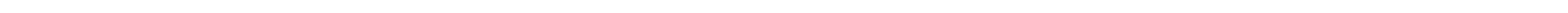
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Appendix



Lamb Grimace Scale Instruction Sheet from Experiment II

Instructions:

The excel file contains 108 sheets with a different lamb face on each page. The pictures are of ‘pre-docking’ and ‘post-docking’ faces and their order has been randomized. For each face, please use the drop down lists to provide:

- A score for each of the action units
- An overall pain score based on your general impression

The scoring scale is described on the following pages. Please become familiar and comfortable with the scale before starting your assessments. Remember to save the file every couple of sheets. When completed, please email the excel sheet, including your name in the title, to: m.j.guesgen@massey.ac.nz

Action Units for scoring the Lamb Grimace Scale

Score each of the action units based on the descriptions provided below. Visual examples are provided on the next page. Please note that the blue dots on the lambs’ faces were used as part of another study and should not impact your assessment, although they may help you determine landmarks on the face.

Orbital Tightening

Lambs in pain show “squeezing” of the eye or closing of the eye, described as orbital tightening. This may only occur, or occur more strongly, in one eye. If the eye closure reduces the visibility of the eye by more than half, it would be scored as severe (2).

Nose features

The nose of lambs in pain appears tightened with a decrease in nostril size. Tightening may be depicted through flattening or ‘pointing’. Flattening makes the nose appear more like a horizontal line in frontal headshots, whereas pointing makes the nose appear more ‘V’ rather than ‘U’ shaped in frontal headshots.

Mouth features

The lips of a lamb in pain are flattened and tightened. The lips appears more like a horizontal line in frontal headshots. There is lack of the ‘upwards curl’ at the edge of the lips that gives lambs their ‘smiling’ appearance when not in pain.

Ear posture

Lambs in pain have ears that are tense and point backwards or downwards so that you cannot see the inner part of the ear. As a result, ears may appear narrower, and flattened dorsally. When lambs are not in pain, their ears are relaxed and horizontal, or slightly forward of the head and you can see the inner ear. Note, please use discretion when scoring lambs where the ear posture is obscured by leaning against objects, eg. pen wall.

Cheek flattening

Lambs in pain show less bulging of the nose and cheek area. In severe cases, the cheek has a 'hollowed' appearance. When not in pain, the cheeks appear rounded in frontal headshots.

Overall Pain Score:

The overall pain score is assigned according to the scale, based on your general impression.

Visual Examples

0- Not Present	1- Moderate	2- Severe
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Orbital Tightening



Nose Features



Mouth Features



Ear Posture



Check Flattening



A note on the previous images: It was difficult to maintain the same camera angle as the lamb was moving in the pen. This may have affected observers' interpretation of particular facial features or altered the quantitative measurements of those features. Ideally, we would use some kind of head-mounted system to capture sheep facial expressions so that the camera angle was maintained.