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Lambs show changes in ear posture when experiencing pain

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Abstract

*Ear posture, or the frequency of postural changes, may reflect various emotional states of animals. In adult sheep (*Ovis aries*), 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. The ear behaviour of four to eight week-old lambs (n = 44) was measured before and after tail-docking using a rubber ring. Each lamb was docked and its behaviour recorded while in the company of an observer lamb of similar age; each acted once as focal (docked) lamb and once as observer within the same pair. Lambs were docked in one of two rounds, so that half were docked in their first exposure to the test environment and half in their second exposure. Tail-docking was associated with an increase in the proportion of time spent with ears backward and decreases in the proportion of time spent with ears plane and forward (mean [± SEM]: Backward: pre 0.12 [± 0.04], post 0.56 [± 0.04]; Plane: pre 0.55 [± 0.05], post 0.19 [± 0.05]; Forward: pre 0.27 [± 0.04], post 0.18 [± 0.04]). There was also a significant increase in the number of changes between ear postures after docking (pre 5.63 [± 0.66], post 9.11 [± 0.66]). Over both periods, female lambs held their ears asymmetrically for longer than males (mean of ranks [± SEM] [raw proportion of time]: Females 52.14 [± 3.44] [0.09 (± 0.01)], males 37.54 [± 3.40] [0.05 (± 0.01)]). 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 physical pain in lambs and may be useful for evaluating 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 (*Ovis aries*) pay attention to (Kendrick *et al* 1995, 1996, 2001, 2007; Ferreira *et al* 2004), and display (Vögeli *et al* 2014), different ear postures according to their emotional experience. Veissier *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,b; Stubsjøen *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,b; Stubsjøen *et al* 2009).

Boissy *et al* (2011) went further, to suggest that negative situations can be characterised 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).

Pain is, by definition, a negative emotional experience (Molony & Kent 1997). Changes in ear posture in response to pain have been observed in mice (*Mus musculus*) (Matsumiya *et al* 2012), rats (*Rattus norvegicus*) (Sotocinal *et al* 2011), rabbits (*Oryctolagus cuniculus*) (Keating *et al* 2012), and horses (*Equus caballus*) (Dalla Costa *et al* 2014) as part of a general facial expression for pain in these species. All species evaluated held their ears backward when in pain. To date, no studies have evaluated ear behaviour in response to pain in sheep. Tail-docking has been shown to cause pain in lambs as indicated by various behavioural (Mellor & Stafford 2000; Thornton & Waterman-Pearson 2002; Grant 2004) and physiological

Table 1 The number of male, female, singleton and twin lambs included in the analysis. This number excludes six lambs that were out of camera view for the entire 30-s sampling period and the six lambs for which sex was not recorded.

Sex	Singletons	Twins	Total
M	11	12	23
F	11	10	21
Total	22	22	44

responses (Lester *et al* 1996; Jongman *et al* 2000; Johnson *et al* 2009). The aim of this study was to see whether ear postures changed during the experience of pain in lambs. We hypothesised that the pain associated with tail-docking would result in an increase in the time lambs spent with their ears held backward as well as an increase in the frequency of ear-posture changes.

The current hypothesis was tested using lambs that were part of a larger study exploring the effects of social environment on the expression of pain behaviour (Guesgen *et al* 2014). 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 or 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).

Materials and methods

As part of the wider social environment study, each lamb was tail-docked and its behaviour recorded while in the company of an observer lamb of similar age; the relatedness and familiarity of the observer lamb varied (ie twin, unrelated but familiar, or unrelated and unfamiliar). The focal lamb's relationship to its partner during testing was included as a factor in the statistical model (see *Statistical analysis*) but was found to have no effect on ear behaviour, thus it is not discussed further. Guesgen *et al* (2014) report the effects of social environment (ie lamb relationship) on other validated pain behaviours.

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. We intended to include 45 mixed-age Romney cross ewes and 78 of their single and twin lambs in this study. However, before commencement of the trial, 13 lambs died from starvation and/or exposure. Subsequently, nine additional lambs had to be excluded because their twin had died. Thus, 56 lambs were available to be tested. The numbers of male and female lambs and singletons and twins tested were roughly balanced (Table 1). This rate of lamb mortality (17%) is within the normal range for extensively kept sheep in New Zealand (eg deNicolo *et al* 2014); however, in future, we would intervene to reduce the number of lambs dying from causes such as inclement weather. There was no prior estimate of variance upon which to base a power analysis and numbers were based on pregnancy scanning results in the experimental flock. Prior to lambing and in the four weeks prior to testing, the ewes and lambs were kept on pasture according to normal New Zealand (NZ) 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 groups based on the social environment the lamb would encounter during testing. 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, social 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 social group and kept in these paddocks for four weeks before the first round of testing began.

Experimental set-up

Tail-docking and observations of behaviour were undertaken in a barn with dirt floors. As noted above, each focal lamb was docked and its behaviour recorded while in the company of an observer lamb of similar age. Each lamb was exposed to the test environment twice, once as the focal lamb (ie tail-docked) and once as the observer (not tail-docked); lambs were kept in the same pairs for both exposures. When only one lamb of a twin set was to be tested (ie in the familiar/unrelated and unfamiliar/unrelated social groups), the focal lamb was randomly selected and the other was never tested. During testing the non-tested twin or twins were kept with their dams. Lambs were docked in one of two rounds, so that half 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. Round two started six days after the end of round one, when lambs were six-weeks old. Pairs were re-tested in the same order as round one.

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 m^2 pen. Their dams were held together in an adjacent 4 m^2 pen and 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.

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Once inside the test pen, one of the lambs was randomly selected and a dot painted on its shoulder to denote that it would be docked (focal lamb). A video camera (Sony Handycam DCR-SR20, Sony Electronics Asia Pacific Pte Ltd, Singapore) 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 before tail-docking (pre-docking period). One researcher then entered the pen and restrained the focal lamb to allow another experimenter to apply the docking ring.

Tail-docking was carried out in accordance with standard NZ husbandry practice. According to the NZ Animal Welfare (Painful Husbandry Procedures) Code of Welfare (Anon 2005), tail-docking without pain relief should be performed when the lambs are as young as possible and not older than six months of age. In this study, lambs were docked at four to eight weeks of age because of the risk of mis-mothering when extensively kept flocks are brought into yards with young lambs and also to allow time for lambs to become familiar with each other to facilitate testing of variation in social environment on pain behaviour.

Docking, which is always performed on lambs in NZ, was undertaken using a rubber ring as this has been shown to cause less acute pain than other methods (Lester *et al* 1996). 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. In future, we would provide analgesia to docked lambs after behavioural observations were complete (ie 30 min after ring application) to relieve any residual pain.

Analysis of focal lambs' ear behaviour

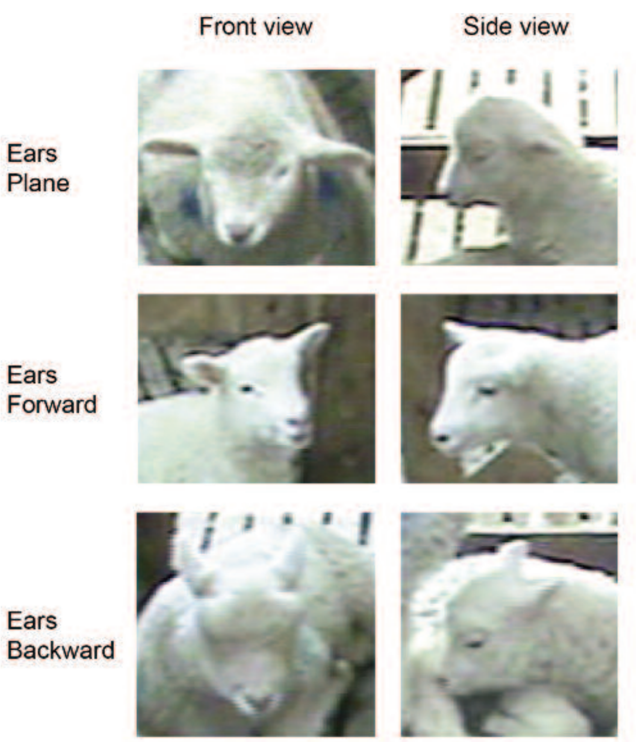
The ear behaviour of focal lambs was scored for 30 s, half-way through each recording period (ie at 15 min before docking and at 15 min after docking). The post-docking sample was taken 15 min after docking as this is when the peak frequency of other pain-related behaviours occurs when rubber rings are 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 (Reefmann *et al* 2009a,b; Stubsjøen *et al* 2009; Veissier *et al* 2009; Boissy *et al* 2011).

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 focal 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 analysed. Six out of 56 focal lambs were out of view for the whole duration of one of the video recordings. A further six lambs were excluded as their sex was not recorded. This meant that their data for both the pre- and post-docking periods were excluded from the analysis (final n = 44; Table 1).

Table 2 Ear-related behaviours scored for actor lambs, based on Reefmann *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 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



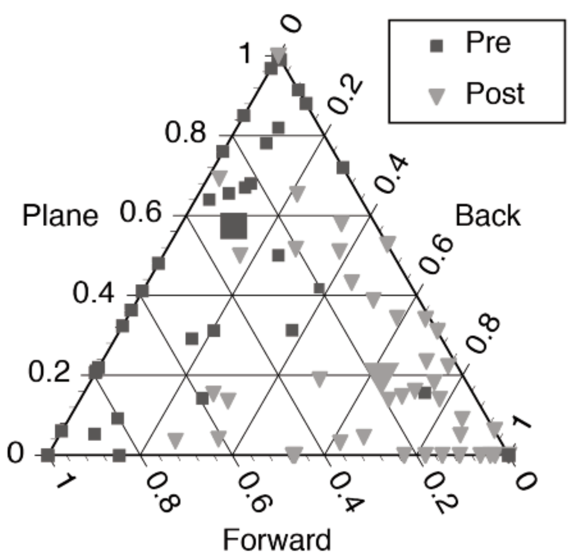
Visual examples of the ear postures scored.

Table 3 Results of MIXED model analysis on frequency or duration of actors' behaviour before and after tail-docking.

Behaviour	Period		Sex		Round		Social environment		Social environment × Round	
	$F_{1, 43}$	*P*-value	$F_{1, 37}$	*P*-value	$F_{1, 37}$	*P*-value	$F_{2, 37}$	*P*-value	$F_{2, 37}$	*P*-value
Ears plane	34.09	**< 0.0001**	1.29	0.26	0.45	0.51	0.62	0.55	0.60	0.55
Ears forward	4.04	**0.05**	2.78	0.10	2.85	0.10	1.19	0.32	0.50	0.61
Ears backward	70.11	**< 0.0001**	1.60	0.21	2.18	0.15	0.59	0.56	0.23	0.79
Ears asymmetrical	1.27	0.27	8.68	**0.006**	3.22	0.08	1.64	0.21	2.59	0.09
Ear changes	18.38	**0.0001**	1.03	0.32	0.78	0.38	1.44	0.25	1.84	0.17

Bold text denotes a statistically significant result at $P < 0.05$.

Figure 2



Ternary diagram summarises the changes in proportions of time spent with ears in each of three postures that changed significantly after docking: ears backward, forward and plane. Small squares indicate the proportion of time spent by individual lambs in each posture before docking while small triangles indicate the proportion of time spent in each posture after docking. The average proportion of time spent in each posture before docking is depicted by a large square and after docking by the large triangle. For each posture, 100% of the time spent in the posture is indicated at one corner (labelled 1.0) and 0% at the other. To read the proportion of time in each posture, begin at the point of interest and follow the diagonal line back to the axis for that particular posture.

Statistical analysis

All statistical analyses were performed using SAS Version 9.2 (SAS Institute Inc, North Carolina, USA). Data were tested to see whether they met the assumptions for parametric analysis; raw data were analysed for time spent with ears forward, back and plane and for number of ear changes. Data for time spent with ears asymmetrical could not be appropriately transformed and thus ranked data were analysed for this variable. A MIXED model was used to evaluate the effects of tail-docking on ear-related behaviours with period (pre- and post-docking) as the repeated measure, lamb within (social environment by round) as the random effect and round (1, 2), focal lamb sex (M, F) and the focal lamb's relationship to its test partner (social environment: twin, familiar/unrelated, unfamiliar/unrelated) and the interaction between social environment and round as fixed effects. A range of models were tested, including all fixed effects (round, sex, social environment) and their interactions, and the model which best fit the data was chosen using the Akaike information criterion. Differences were considered significant at $P < 0.05$.

Results

There was a significant effect of period on all behaviours except time spent with ears asymmetrical (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 and forward. Figure 2 summarises the changes in the proportion of time lambs spent with their ears back, forward and plane from the pre- to post-docking period. There was also a significant increase in the number of changes between ear postures from pre- to post-docking (mean [± SEM]: pre 5.63 [± 0.66], post 9.11 [± 0.66]).

Sex had a significant effect on the proportion of time spent with ears asymmetrical. Over both periods, female lambs spent more time holding their ears asymmetrically than males (mean of ranks [± SEM] [raw proportion of time]: Females 52.14 [± 3.44] [0.09 (± 0.01)], males 37.54 [± 3.40] (0.05 [± 0.01)]).

Discussion

This study investigated whether, and how, ear posture changes in lambs when they are experiencing physical pain. As expected, there was a significant effect of tail-docking on ear behaviour. Tail-docking was associated with an increase in the time spent with ears held backwards and a decrease in the time spent with ears held forwards or in the plane/axial position. The number of changes between ear postures also increased after docking. The same lambs also showed significant changes in validated pain-related behaviours, such as increased activity and time spent in abnormal postures and decreased time spent in normal postures after docking (Guesgen *et al* 2014). Therefore, we can infer that any concurrent change in ear posture after docking was related to the experience of pain.

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It is important to note that while changes in four separate ear postures are discussed here, these postures are not independent of each other. If the proportion of time spent in one posture increases, then the total proportion of time spent in the other three postures must decline. However, how the other three change and to what extent can only be revealed by separate statistical analysis of each. If each posture means something different in terms of the animal's emotional experience, as is suggested by previous studies, then information on the direction and extent to which each posture changes due to pain is useful — even if this is as simple as defining negative versus positive experiences. For example, isolation has been associated with a forward ear posture whereas rumination (indicative of a neutral or positive state) has been associated with the plane ear posture (Reefmann *et al* 2009a,b; Stubsjøen *et al* 2009). In the current study, pain appears to be associated with a pattern of change consisting of an increase in the time spent with ears in a backward ear posture and less time spent in the plane and forward postures. It remains to be investigated whether the degree of pain is related to the extent to which ear behaviour of lambs or other animals changes.

Our findings are consistent with studies of other species, such as horses and rabbits, in which pain elicited backward ear postures (Keating *et al* 2012; Dalla Costa *et al* 2014). Similarly, negative situations, such as physical capture and a stressful social learning task were found to elicit backward ear postures in silver foxes (*Vulpes vulpes*) and cattle (*Bos taurus*) (Moe *et al* 2006; Coulon *et al* 2011). Our findings also support the theory that the perceived controllability, or lack thereof, of a negative situation can influence ear posture (Boissy *et al* 2011). The cause of docking pain was likely perceived to be uncontrollable by lambs, which may explain the increase in the backward ear posture.

Lambs also spent less time with their ears in the plane posture after docking. In adult sheep, ears held in the plane/axial posture has previously been associated with emotionally positive situations, such as rumination or standing in the feed area (Reefmann *et al* 2009a,b; Boissy *et al* 2011). Thus, this posture likely reflects the absence of strong negative emotions, and it makes sense that the time spent with ears in this posture would decline during significant pain, such as occurs after tail-docking.

Also consistent with previous studies of negative emotion, tail-docking was associated with an increase in the number of ear-posture changes (Reefmann *et al* 2009a,b, 2012). This increase may reflect the conflicting motivations of the animal. After docking, the lamb was holding its ears in a backward posture; however, it would still be motivated to remain alert to its surrounding environment which also requires ears to be held forward. Hence, the frequency of change between these postures could represent the strength of the motivations to be vigilant and to display a pain-related behaviour as a correlate to the sensation of pain or as a potential communicative tool. Another interpretation of these results is that an increase in ear-posture changes due to docking reflects anxiety.

While our findings are similar in some respects to those of previous studies investigating ear-posture changes in sheep, there is one key difference. In the current study, the time spent with ears held forward decreased after docking, suggesting that ears forward does not reflect the negative emotional state of pain. In contrast, several previous studies interpreted the forward ear posture as indicative of negative emotion, elicited by separation from other group members (Reefmann *et al* 2009a,b; Stubsjøen *et al* 2009). However, separation from the flock will elicit increased alertness or arousal as the sheep tries to gather sensory information to allow it to reunite with the group. Thus, while separation may be accompanied by negative emotion, ears forward may be a specific behavioural strategy to rectify the problem. In our study, lambs could stay in close visual and physical proximity to their dams and siblings and thus spent only about 30% of the observed period before docking with their ears forward, probably in response to changes in the behaviour of other lambs and ewes in the vicinity. After docking, the lambs were probably distracted from the social environment by the experience of pain, resulting in the decrease in time with ears held forward. Along similar lines of reasoning, vigilance may decrease from before docking to after docking as a result of the lamb's attention being more directed towards the sensation of pain. Similarly, as time went on, the lamb would become familiar with its test environment and also display less vigilance, in the form of less time with ears spent in the forward posture.

Finally, we found some effect of sex on lambs' ear posture. Females held their ears asymmetrically for longer overall than males. 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 that it might be indicative of some negative emotional experiences. In the present study, the small amount of time spent with ears in this posture (five to nine percent) suggests it represented a transitional posture when ears were being moved from one posture to another. The observed sex difference may indicate the greater desire of female lambs to simultaneously attend to more than one relevant stimulus in the environment.

Pain behaviours have various functions, including alleviating pain, facilitating healing and social communication (Allen 2004). Changes in ear behaviour during pain are unlikely to alleviate pain or facilitate healing directly and therefore the most likely advantage for displaying ear-posture behaviours relates to social communication. Paying attention to the behaviour of conspecifics may be particularly useful in social species, such as sheep, as it can provide information that enhances survival (Clayton 1978; Maynard Smith & Harper 2003). The expression of pain via ear postures may act as a cue to other group members, informing them of the sufferer's emotional state (Fox 1971; Williams 2002), and conspecifics may consequently engage in helping or avoidance behaviour. We might therefore expect that the social environment influences the expression of pain via ear postures. For example, animals may be expected to show a greater intensity of pain behaviour in the presence of a related conspecific. This was not found to be the case and therefore it might not be the expression of pain that changes with social environment but the observer's response to that social cue. This remains to be tested.

Animal welfare implications

As well as providing information to conspecifics, ear postures provide a useful cue for welfare assessment. In addition to pain caused by husbandry procedures, such as tail-docking, ear posture could be used as a non-invasive indicator of pain due to diseases, for example foot rot and mastitis.

Conclusion

This is the first study to demonstrate changes in the ear posture of lambs associated with the experience of pain. The possibility of using ear posture as a non-invasive welfare indicator is an avenue for further investigation. To further validate ear postures as an indicator of the negative emotion of pain, analgesia should be provided to abolish changes after docking. It would also be interesting to see whether the degree of pain is related to ear behaviour of lambs.

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