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Ear and tail postures as indicators of emotional valence in sheep☆

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ABSTRACT

To date, most studies on animal emotions have focused on the assessment of negative emotional states, and there is a lack of approaches to characterising positive emotional states. The aim of this investigation was to measure differences in ear and tail postures in sheep exposed to situations likely to induce states of negative, intermediate and positive emotional valence.

Nineteen female sheep were observed in emotion-eliciting situations in two experiments. In the home-pen experiment, ear and tail postures were observed during separation from group members (negative situation), during rumination (intermediate), and while feeding on fresh hay (positive situation). In the fodder experiment, individual sheep were conditioned to anticipate the delivery of standard feed. Once familiar with this experimental condition, they were offered either the standard feed (control treatment), unpalatable wooden pellets (negative treatment), or energetically enriched feed mixed with preferred feed items (positive treatment). Ear and tail postures of sheep were recorded during the final 6 min preceding feed delivery (anticipation phase) and for 6 min during feed delivery (feeding phase). Data were analysed using linear mixed-effect models.

In the home-pen experiment, sheep separated from group members showed a high number of ear-posture changes and a high proportion of forward ears compared to hay feeding, during which ears were mainly passive. In the fodder experiment, the total number of ear-posture changes was generally high during the anticipation phases, slightly lower during delivery of the wooden pellets, and clearly reduced during the delivery of standard and enriched feed. A higher proportion of passive ear postures occurred when standard feed and enriched feed were offered compared to the delivery of wooden pellets. The proportion of asymmetric and axial ear postures was influenced by the sequence of testing of the different feeding treatments, with a higher proportion of asymmetric and a lower proportion of axial ear postures during the first exposure to either the wooden pellets or the enriched feed. A high proportion of the sheep's tails being raised was only observed during separation from group members.

In both experiments, frequent ear-posture changes were most clearly associated with situations inducing negative states, and a high proportion of passive ear postures with situations likely to induce positive emotional states. Unfamiliarity influenced emotional reactions towards a more negative appraisal. A raised tail only appears to occur in specific situations, and was not useful for distinguishing emotional valence. Apart from the need for further validation, observations of ear-posture changes seem to be a promising approach for assessing emotional reactions in sheep.

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1. Introduction

Recent definitions of good animal welfare emphasise the presence of positive emotions in addition to the absence of negative emotional states (Broom, 1991; Duncan, 1993, 2005; Boissy et al., 2007). The subjective nature of short-term emotional states as well as longer-term mood, however, makes their objective assessment difficult (Curtis and Stricklin, 1991; Rushen, 1996). Developing approaches to measure emotional states as objectively as possible, particularly by non-invasive and easily applied methods, remain a scientific challenge (Edwards, 2007; Winckler et al., 2007). The lack of approaches to measuring positive emotions and good animal welfare is especially pronounced (Boissy et al., 2007).

Behaviour patterns typically observed for the assessment of emotional valence, such as approach behaviour to positive stimuli and withdrawal behaviour or flight distance as a response to negative emotional stimuli (Hargreaves and Hutson, 1990; Vandenheede et al., 1998), cannot be used to judge situations from the viewpoint of an animal whose movement is restricted. Other behaviour patterns such as eliminative behaviour occur infrequently, and are not shown consistently across individuals and situations (Fitzpatrick et al., 2006). Consequently, more sophisticated and frequently occurring behaviour patterns that can be observed in a wide variety of situations must be found (Veissier and Boissy, 2007).

In humans, mimics reflect experienced emotions well (Bradley and Lang, 2000). Facial motor patterns are universal across cultures (Ekman and Friesen, 1971), and it is difficult to suppress emotional expressions since they are automatic and highly stereotyped (Fridlund, 1991). In most animals, including sheep, facial expressions would appear hard to interpret – at least from a human point of view. Nevertheless, facial expressions relating to gustatory sensory pleasures have been found to occur in rats as well as in humans (Berridge, 2003), and palatability can be reliably interpreted since positively and negatively rated tastes are accompanied by distinct motor patterns (Berridge, 2000). Even though sheep do not appear to have a wide array of facial expressions, ruminants have several muscles for rotating their ears (Nickel et al., 1968), and there are indications that ear postures may be useful in assessing emotional valence in animals. In cattle, for example, a high occurrence of pendulous ear postures was used as an indicator of the animals' positive rating of their favourite grooming sites (Schmied et al., 2008). In sheep, the duration of pricked or asymmetric ear postures differs depending on the cognitive appraisal of environmental stimuli (Désiré, 2004). Moreover, asymmetry of ear postures may differ with negative and positive emotional states, due to lateralised behaviour patterns (Quaranta et al., 2007) thought to result from contra-lateral hemispheric brain activity (Davidson, 1992; Wager et al., 2003). Another potential behavioural indicator of emotional valence may be tail posture, used to measure pain or stress in calves and lambs (Graf and Senn, 1999; Grant, 2004).

The aim of this study was to investigate whether ear and tail postures are useful for characterising the valence of emotional states in freely moving sheep. In two experiments, animals were subjected to representative situations likely to induce states of negative, intermediate, and positive emotional valence. In the home-pen experiment, ear and tail postures were observed during separation from group members as a negative situation, during rumination as an intermediate situation, and while feeding on fresh hay as a positive emotional situation. In the fodder experiment, the same sheep were subjected to feed-related positive–negative contrasts in which they were trained to anticipate the supplying of standard feed. Once they were familiar with this experimental condition, their feeding expectations were either fulfilled by offering the familiar standard feed, frustrated by offering unpalatable wooden pellets, or surpassed by offering energetically enriched feed mixed with preferred feed items. Ear and tail postures were recorded preceding feed delivery (anticipation phase) and during feed delivery (feeding phase).

2. Animals, materials and methods

2.1. Animals, housing and husbandry

Nineteen non-lactating female sheep (9 Swiss White Alpine and 10 Lacaune ewes) were acquired at about 4 months of age (November 2005 and February 2006) from a shepherd who had raised them all as lambs. They were housed as a group at the Agroscope Reckenholz-Tänikon Research Station ART (Tänikon, Switzerland) in an open-front pen (58 m^2) consisting of an area with deep-litter straw bedding (42 m^2) and a feeding area with a solid concrete floor and a 7 m-long hayrack. When weather conditions were dry, the animals were given access to an additional outdoor exercise yard (18 m^2). From spring to autumn, sheep were on pasture at times when no experiments were being conducted. Water and hay were available ad libitum and the hayrack was refilled twice daily at around 8:30 and 16:00.

2.2. Experimental designs

In the home-pen experiment, each of the 19 sheep was observed for 5 min in three situations – separation from group members, rumination, and feeding on fresh hay – of negative, intermediate, and positive emotional valence, respectively. These situations might have provoked states potentially similar to specific emotions like anxiety, satisfaction, and elation in humans. The experiment was conducted during November 2006 in the animals' home pen. For the separation from group members, a given focal sheep was separated from the herd and kept in the litter area, while all other sheep were moved to the outdoor exercise yard and out of sight of the focal animal. Rumination was observed from 2 h after hay feeding in the morning until the afternoon refill, while the focal sheep was standing in the deep litter or the feeding area. Feeding of a focal animal was observed for the first 15 min after provision of fresh hay in the rack. According to a breed-balanced schedule, each focal sheep was observed twice in each of the three situations.

The animals' behaviour was directly observed from outside the pen by two experimenters, using focal-animal sampling and instantaneous recording (Martin and Bateson, 1993) at 15-s intervals for a total of 5 min (21 sample points). One observer recorded the focal sheep's tail posture (whether raised by more than 10° from the perpendicular to the backbone or not), while the other recorded the following ear postures, separately for the right and left ear: axial ear (perpendicular to the head-rump axis), forward ear (tip of the ear towards the front at an angle of more than 30° from the perpendicular), backward ear (tip of the ear towards the back at more than 30° from the perpendicular), auricle forwards (opening of the ear auricle facing towards the front), auricle down (opening of the ear auricle facing towards the ground) and passive ear (ear hanging down loosely and dangling along with any head movement). In addition, the total number of ear-posture changes per observation time (at least one ear is moved from forwards, axial, or backwards to another of these postures from one sampling point to the next), the proportion of asymmetric ears per observed sample points (right and left ear in a different posture) and the proportion of left-lateralised ears (number of times when the *left* ear is forwards and the right ear is axial or backwards, divided by the sum of these postures plus those when the *right* ear is forwards and the left ear axial or backwards) were calculated from the recorded data.

In the fodder experiment, the same sheep were subjected to feed-related positive–negative contrasts, in which feeding expectations were either fulfilled by offering the familiar standard feed, frustrated by offering unpalatable wooden pellets, or surpassed by offering energetically enriched feed mixed with preferred feed items. These situations might have provoked states potentially similar to specific emotions such as disappointment, satisfaction, and joy in humans, respectively. This experiment was carried out from May to June 2007 in a building near the sheep's home pen. During data recording, three sheep (one focus and two companion sheep) were kept singly side-by-side in three partitions measuring 0.9 m × 2.40 m each. The walls between the partitions were of wire netting, enabling the animals to remain in visual contact with each other. At the front end of each partition was a feed trough, which could be filled with different types of pellets by means of an automatic feeder. Data was collected during a 6-min period prior to feed delivery (anticipation phase) and a 6-min period coinciding with feed delivery (feeding phase).

The sheep were habituated to the experimental procedure. During the first 2 weeks, they were trained to voluntarily walk into the building with the three partitions every day, first in groups of several animals and later in triads (each sheep visiting the test room 4–10 times). Over the next 2 weeks, triads were conditioned to anticipate being fed the standard feed (UFA 250 pellets, NEL 4.0 MJ/kg) with a delay of up to 6 min. To achieve this, each focal sheep underwent training on 7 days, with three trials per day. During this period, the length of the anticipation and feeding phases was increased from 1 to 6 min in 1-min steps.

For each experimental trial, the passage to the experimental room was opened so that sheep voluntarily entered the test room. The focal animal was gently moved into the middle partition, flanked in the partitions on either side by two companion sheep not used as focal animals themselves. After a 5-min rest period allowing the animals to calm down from their walking, the experiment began with the anticipation phase: in each partition, a green light bulb at the height of the sheep's head lit up, and the engine of the automatic feeder began to run for 6 min without dispensing feed. The green light changing to red ushered in the feeding phase, in which feed was continuously dispensed into the three troughs for 6 min. On 3 consecutive days and at the same time of day, each sheep was exposed to feeding treatments assumed to differ in terms of emotional valence. On the first day of testing, the standard feed (400 g) was dispensed into the troughs (standard-feed treatment). Data collected during the anticipation phase of this day served as a baseline for describing the behaviour of a sheep expecting nothing but delivery of the standard feed. On the second and third days of testing, balanced for breed and age, half of the animals were first tested in a treatment assumed to have negative valence, the other half in a treatment assumed to have positive valence for the sheep. In order to provoke a negative emotional state, sheep were given wooden pellets (400 g) instead of the standard feed (wooden-pellet treatment). To induce a positive emotional state, a mixture of energetically more valuable feed (UFA 864 pellets, NEL 7.0 MJ/kg), raisins and small pieces of dry bread (400 g in total) was dispensed into the troughs (enriched-feed treatment).

A Panasonic WV-CP450 camera was placed above the end of the middle partition to record the behaviour of the focal sheep from a top-back view. Images were observed directly on a monitor in a box behind the partitions, but with the observer (NR) out of sight of the animals. Forward, axial and backward ear postures, and whether these were passive (for definitions see above) were recorded continuously and separately for the sheep's right and left ears by means of the Etho data-collection software (© Dr. R. Weber; Agroscope Reckenholz-Tänikon Research Station ART, Tänikon). The definitions of all ear-posture measurements were adopted from the home-pen experiment (see above). Based on continuous recordings during 6 min, the total number of active ear-posture changes was calculated (without counting ear postures with passive ears), and all ear-posture measurements (e.g. asymmetric and left lateralised) were calculated as proportion of all observed ear postures. Tail posture was recorded and analysed by instantaneous recording every 15 s (25 sample points).

2.3. Analysis

Statistical analysis was performed in R (version 2.5.1; R Development Core Team, 2007). Data of the home-pen experiment was summed per individual and across the 21 sample points from each of the two repetitions of the three experimental situations. Since the proportions of both the forward ears and auricles forwards postures, as well as the backward ears and auricles down postures were highly correlated (Spearman; rho $\geq$ 0.8; $p \leq 0.05$), only the results for the proportions of forward and backward ears are presented. Except for the total number of ear-posture changes, variables are given as proportions. Each ear variable as well as the proportion of sample points with a raised tail were modelled separately using linear mixed-effects models (Pinheiro and Bates, 2000), with situation (separation from group members, rumination, feeding on fresh hay) as an explanatory factor. In order to reflect the experimental design, a random effect for repetition nested in sheep was included. The assumptions of normal distribution and homoscedasticity of errors of the models were checked by a graphical analysis of the residuals. Logit transformations were conducted for the proportions of asymmetric and left-lateralised ear postures, and the proportion of raised-tail postures to account for deviations from the model assumptions. Due to large differences in the variance of data between the situations, a term accounting for this heteroscedasticity was included for the proportions of backward ears and raised-tail postures.

Data from the fodder experiment was summed per individual, separately for the 6 min of the anticipation phase and the 6 min of the feeding phase for each of the three experimental trials. Due to software failure, data were available for analysis for only 14 animals. All ear postures were modelled as response variables using mixed-effects models that included the explanatory variables type of trial (wooden pellets, standard feed, enriched feed), phase (anticipation, feeding) and sequence of testing (standard feed–wooden pellets–enriched feed or standard feed–enriched feed–wooden pellets), as well as all possible interactions. Except for type of trial, phase and their interaction, the terms were reduced in a step-wise backwards procedure and will only be mentioned if significance was reached ($\alpha \leq 0.05$). To account for dependency in the data set, a random effect for experimental trial nested within individual sheep was included. Assumptions of these models were checked as described above and transformations were used for the number of ear-posture changes (log transformation) as well as for the proportions of backward, passive, asymmetric and left-lateralised ears (logit transformation). Raw data are presented as boxplots indicating observed median, first and third quartile, and absolute range of data. All mean values given in the text are based on model estimates.

3. Results

3.1. Home-pen experiment

In the home-pen experiment (Fig. 1), the total number of ear-posture changes was highest during separation from group members (estimated mean: 11 changes), decreased during rumination (9 changes) and was lowest during feeding on fresh hay (4.5 changes; Fig. 1a, $F_{2,73} = 25.31$; $p < 0.001$). Sheep showed similar proportions of asymmetric ear postures during separation (0.18) and rumination (0.18), and reduced proportions during feeding on fresh hay (0.10; Fig. 1b; $F_{2,73} = 8.74$; $p < 0.001$). The proportion of forward ears was highest during separation (0.65; Fig. 1c). This proportion decreased during rumination (0.11), and almost never occurred during feeding (0; $F_{2,73} = 267.92$; $p < 0.001$). An opposite and more linear pattern was found for the proportion of backward ears (Fig. 1d; separation = 0.07; rumination = 0.54; feeding = 0.89; $F_{2,73} = 238.28$; $p < 0.001$). The proportion of passive ears was above zero only during rumination and feeding (Fig. 1e; separation: 0; rumination: 0.49; feeding: 0.45; $F_{2,73} = 42.65$; $p < 0.001$). The proportion of left-lateralised ears did not differ between situations (separation = 0.41; rumination = 0.48; feeding = 0.48; $F_{2,60} = 0.45$; $p = 0.64$).

The proportion of sample points with raised tail was high during separation (0.68) and much lower during rumination (0.01) and feeding (0.02; Fig. 1f; $F_{2,73} = 156.17$; $p < 0.001$).

3.2. Fodder experiment

Anticipation phases in the fodder experiment did not differ significantly for any of the response variables (Fig. 2). The total number of ear-posture changes observed continuously for 6 min was highest during anticipation (86–98) and slightly lower during the wooden-pellet treatment (82), while the fewest ear movements were observed during the standard-feed (15) and enriched-feed treatment (17; Fig. 2a; interaction of type of trial and phase: $F_{2,37} = 20.47$; $p < 0.001$). The proportion of asymmetric ear postures (Fig. 2b) showed a pattern similar to that observed for the number of ear-posture changes (anticipation: 0.30–0.52; wooden pellets: 0.24–0.39; standard feed: ~0.07; enriched feed: 0.05–0.12; three-way interaction of type of trial, phase, and sequence of testing: $F_{2,33} = 5.58$; $p = 0.008$). In the wooden-pellet treatment, ears were more asymmetric if wooden pellets were tested before enriched feed (solid line) than if enriched feed was tested before wooden pellets (dotted line). In the enriched-feed treatment, ears were less asymmetric if wooden pellets were tested before enriched feed. Hence, there was an increase in the proportion of asymmetric ear postures in the first treatment following the standard-feed treatment, irrespective of whether wooden pellets or enriched feed were offered.

The increase or decrease in the proportion of forward ears from the anticipation to the feeding phase did not differ between treatments (Fig. 2c; interaction of type of trial and phase: $F_{2,37} = 1.80$; $p = 0.18$). The proportion of backward ears was generally low (<0.17), but decreased in the wooden-pellet (0.11) and the enriched-feed treatment (0.10) compared to the standard-feed treatment (0.16; interaction of type of trial and phase: $F_{2,37} = 3.54$; $p < 0.04$).

The proportion of axial ears (Fig. 2d) showed an inverse pattern to that for asymmetric ears. Their proportion was generally low during the anticipation phases (0.06–0.13), slightly higher during the wooden-pellet treatment (0.13–0.24), and highest in the standard-feed (0.48–0.51) and enriched-feed treatments (0.43–0.55; three-way interaction of type of trial, phase, and sequence of testing:

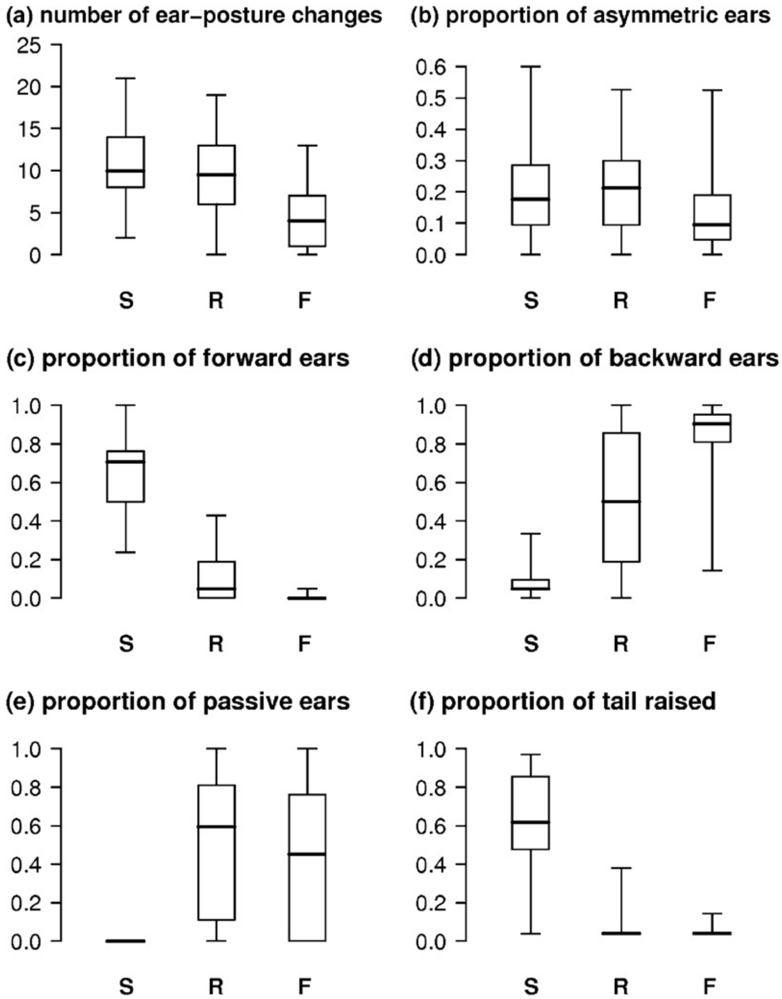

Fig. 1. Ear and tail postures observed in sheep ($n = 19$) exposed to three situations (5 min each, resulting in 21 sample points per situation) of different emotional valence in the home-pen experiment: separation from group members (S: negative valence), rumination (R: intermediate valence) and feeding on fresh hay (F: positive valence).

$F_{2,33} = 4.16$; $p = 0.02$). Thus, the first treatment following the standard-feed treatment was associated with a lower proportion of axial ears, irrespective of whether wooden pellets or enriched feed were offered. The pattern for the proportion of passive ears (Fig. 2e) was similar to that observed for the proportion of axial ears, with highest values during the standard-feed (0.28) and the enriched-feed treatment (0.21; anticipation: ~0.01; wooden pellets: 0.03; interaction of type of trial and phase: $F_{2,37} = 8.73$; $p < 0.001$).

The proportion of left-lateralised ears differed depending on treatment (Fig. 2f; interaction of type of trial and phase; $F_{2,27} = 5.15$; $p = 0.01$), increasing from the anticipation to the feeding phase in the wooden-pellet trial (+0.08), decreasing from the anticipation to the feeding phase in the standard-feed trial (−0.19), and increasing slightly from the anticipation to the feeding phase in the enriched-feed trial (+0.03).

The sheep's tails always hung down, both during anticipation as well as during all feeding phases.

4. Discussion

Ear postures of sheep in both experiments allowed for clear differentiation of the experimentally induced situations of assumed negative and positive valence (separation from group members versus feeding on fresh hay in the home-pen experiment, and delivery of wooden pellets versus enriched feed in the fodder experiment). During negative situations, the number of ear-posture changes, and the proportion of forward and asymmetric ear postures were high, whereas axial and passive ear postures rarely occurred. By contrast, positive situations were characterised by few posture changes, a low proportion of asymmetric and a high proportion of passive ear postures.

Both experiments showed that negative emotional states are characterised by a high number of ear-posture changes, whereas positive emotional states would seem to be accompanied by passive ears. In the home-pen experiment, ears were mainly backwards during hay

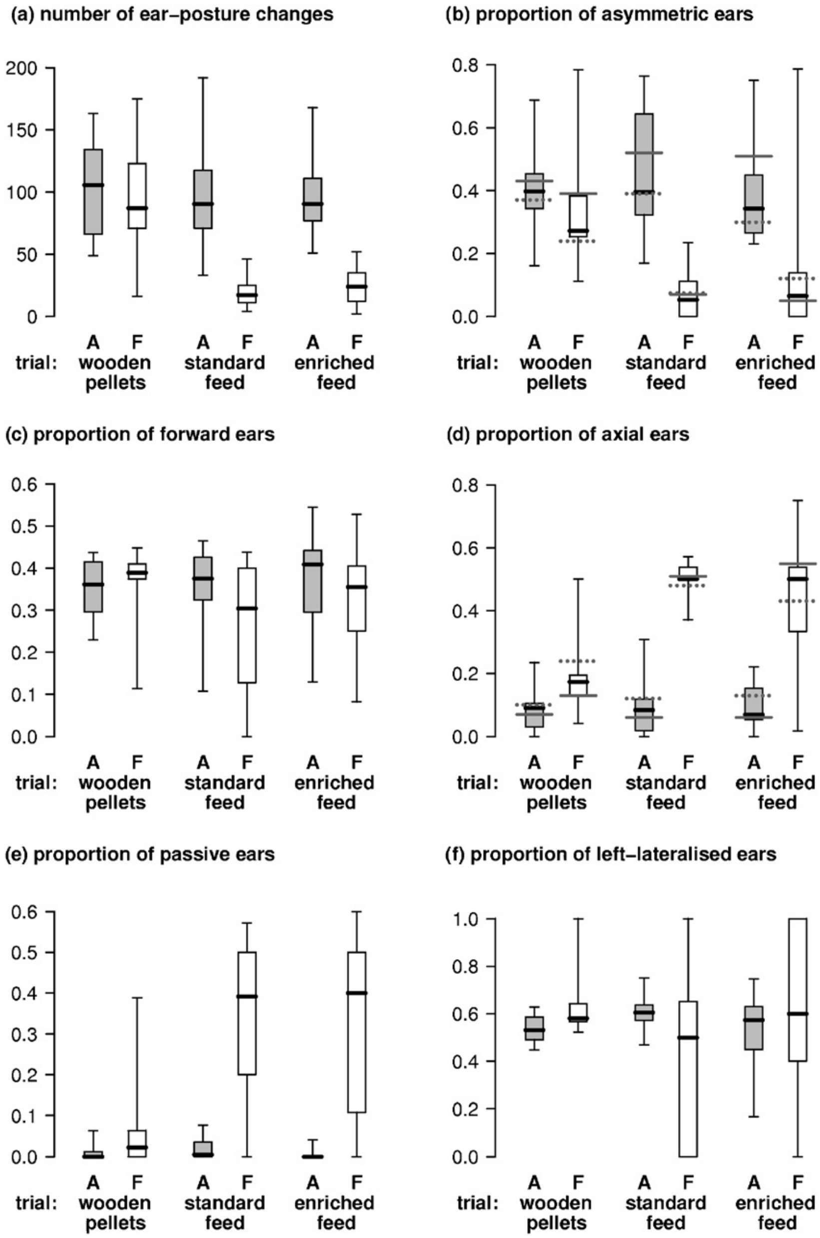

Fig. 2. Ear postures observed continuously in sheep (n = 14) during the anticipation (A, in grey) and feeding (F, in white) phases (6 min each) in the fodder experiment when offered wooden pellets (treatment of negative valence), standard feed (control treatment) or enriched feed (treatment of positive valence). Lines indicate estimated means for sequence of testing (solid grey: wooden pellets offered before enriched feed; dotted grey: enriched feed offered before wooden pellets).

feeding, as they hung passively from the sheep's uplifted heads. Since passive ears can hang axially, forwards or backwards, depending on head posture, passivity of ears in the fodder experiment was recorded in addition to the actual ear posture. As the recording of passive ears requires high concentration, they may be best observed by low numbers of ear-posture changes in future studies.

Considering that emotional states are subjective (Siemer et al., 2007; Veissier and Boissy, 2007), a priori assumptions were made regarding the animal's perception of the valence of the experimental situations. The fodder experiment was based on successive positive–negative contrasts (Flaherty, 1982) that were expected to elicit a negative emotional reaction when sheep were fed wooden pellets, and a positive emotional reaction when given enriched feed. The standard feed was not expected to provoke a specific emotional contrast reaction since expected and actual reward did not differ. However, from anticipation to the delivery of any feed (either standard or enriched), sheep might have experienced a positive contrast because no feed was available during the anticipation phase but the actual supply of feed thereafter was positive for the animals. It is thus likely that the sheep perceived the standard-feed treatment as positive because they were ultimately fed, and that feeding per se may have been more important than the type of feed, as discussed for rats (Flaherty and Mitchell, 1999). This would then explain the similar reactions of sheep to the standard-feed and the enriched-feed treatment. The feeding of wooden pellets clearly provoked a negative contrast reaction, compared to both the standard- and the enriched-feed treatment, as well as in succession from the anticipation to the feeding phase.

Ears are essential for gathering information from the environment which is then integrated and appraised (Manteuffel, 2006). Negative situations require increased attention to environmental surroundings to allow for problem-solving. When wooden pellets were given, the expectations of the sheep were frustrated; since they had never encountered this situation before, however, they may still have expected to be given feed. When the sheep received the expected reward, behavioural activation in the form of ear activity decreased because attention to external stimuli was no longer necessary. The strong interrelation of attention and emotion, in particular with regard to negative emotional states and high attention (Carretié et al., 2001), indicates that observations of ear postures are not adversely affected by attention, but that attention is an inherent component of an emotional reaction.

During anticipation of feeding as a positive event, attention is increased (Melges and Poppen, 1976) to notice immediately if and when a reward will be given. However, increased attention is also known to occur during anticipation of negative emotional states (Spruijt et al., 2001). In the fodder experiment, all ear-posture variables differed only slightly when comparing the anticipation phases with the wooden-pellet treatment, potentially indicating that it might have been negative for the sheep. The anticipation of positive events may be perceived as uncontrollable and unpredictable due to uncertainty about future events that had not happened at that time (Gordon, 1987), and may change to frustration if the reward is given too late (Couvillon and Bitterman, 1984; Waitt and Buchanan-Smith, 2001). However, silver foxes show similar rates of flat and backward-rotated ears during the anticipation of both positive unpredictable and negative predictable situations (Moe et al., 2006). Since we did not directly compare the response of ear postures to positive and negative anticipatory situations in the fodder experiment, we cannot draw any conclusions of the usefulness of ear postures for distinguishing emotional valence during anticipatory situations. The reactions during appraisal of a positive stimulus (anticipation) may not be the same as during a given positive situation.

Fridlund (1991) speculated that the erectors of the ear auricle in non-human mammals are homologous to the muscles involved in lowering eyebrows in humans. The tension in these eyebrow muscles is higher during negative than during positive emotional states (Witvliet and Vrana, 1995). This would indicate that negative emotional states might coincide with erect ears (in our study forward or active ears), whereas positive emotional states might coincide with non-erect (i.e. passive) ears.

The sheep's tails were raised during separation from group members, but not during rumination or feeding on fresh hay in the home-pen experiment, or in any of the anticipation or feeding phases in the fodder experiment. This might indicate that a raised tail occurs only during intensely negative emotional states. Other studies observed, however, that calves (Scheurmann, 1974) and lambs raise their tails while sucking, and qualitative observations showed that tame sheep raise their tails while being groomed by humans. Assuming that sucking and being groomed are perceived as positive by sheep, a raised tail may indicate strong emotional activation in general, regardless of negative or positive valence.

In the home-pen experiment, the valence for rumination was expected to lie between separation from group members and the supply of feed. Data showed that some ear postures were more similar to those of separation (number of ear-posture changes, proportion of asymmetric ears) and others to those of feeding (proportions of passive and forward ear postures). Owing to practical limitations, ear postures were recorded at every 15 s only, so that some ear-posture changes, particularly during separation, may have been missed. Consequently, the situation of rumination may have been more intermediate or positive than appeared to be the case, and ear postures should be assessed continuously, as done for the fodder experiment. In addition, rumination may not coincide with a strong emotional reaction directly provoked by an environmental stimulus, such as separation or the supply of feed, and the suitability of using ear postures for assessing relatively docile emotional states of longer duration and mood should be investigated.

Emotion involves some form of cognitive appraisal (Dantzer, 2001), which may explain the effect of the sequence of testing of the different types of treatments on the proportions of asymmetric and axial ear postures in the fodder experiment. The sheep's reactions to the enriched-feed treatment were more similar to their reactions to the wooden-pellet treatment if the enriched-feed treatment directly followed the standard-feed treatment. The difference between the situations of assumed negative and positive valence was greater if the treatment representing a negative valence was tested before the one representing a positive valence. Animals were habituated to receiving standard feed, and the pellets offered in the following treatment (either wooden pellets or enriched feed) were different in terms of feed composition, and hence unfamiliar or novel. Supporting these results, Désiré (2004) observed that unfamiliar events were characterised by pricked and asymmetric ears. With additional habituation, pricked ears decreased and more hanging ears occurred in her experiment. Unfamiliar or novel situations seemed to be interpreted at first as potentially negative (Carretié et al., 2001; Herwig et al., 2007) and elicited increased attention (Levine, 2007) in order to gather more environmental information (Dolan, 2002) for judging the situation. Positive but unfamiliar situations may therefore be perceived as less positive than situations that are both familiar and positive. If the treatment representing a negative valence directly followed the standard-feed treatment, the effect of unfamiliarity was superimposed on the negative emotional reaction elicited by the wooden pellets.

As with previous investigations (Wager et al., 2003), support for lateralised behaviour patterns regarding emotional valence was inconsistent across the two experiments. No support for left-lateralised ear postures was found in the home-pen experiment. In the fodder experiment, the proportion of left-lateralised ear postures increased from the anticipation to the feeding phase in the trial of negative valence (wooden pellets) and decreased from anticipation to feeding in the trial with standard feed. Assuming that the standard-feed treatment was actually positive for the animals (as discussed above), treatments representing negative and positive valence might be distinguishable by the proportion of left-lateralised ear postures. Against our expectations, their proportion increased slightly in the enriched-feed trial, which may, however, be explained by the unfamiliarity of the situation, as discussed above for the sequence of testing. Lateralised ear postures might thus help to perceive emotional valence from a sheep's perspective after further validation.

So far, observations of ear postures appear to be a good non-invasive tool for assessing the valence of emotional states in sheep, assuming that experiments directly compare the relative valence of different situations (Broom, 1991). Nevertheless, validation of these ear variables in additional situations and in parallel with more established physiological measures of emotional states is needed. The main advantage of ear postures over physiological measures as an indicator of emotional states is that the former are less likely to be influenced by diurnal physiological fluctuations or physical activity, in contrast to, e.g. glucocorticoids (Fulkerson and Tang, 1979) or heart rate (Baldock et al., 1988; Chan et al., 2007).

5. Conclusion

Negative emotional states appear to coincide with a high number of ear-posture changes, and positive emotional states with a high proportion of passive ear postures. Although further validation is needed, observing ear postures may be a useful approach for assessing emotional reactions of sheep during experimental situations.

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