Housing induced mood modulates reactions to emotional stimuli in sheep

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ABSTRACT

The assessment of positive and negative short-term affective states (emotions) in animals and their modulation by long-term affective states (mood) is an on-going challenge. This study investigated the use of behavioural and physiological measures to assess emotions and their modulation by mood in sheep. To induce a more Positive and more Negative mood, two groups of sheep ($n = 10$ and 9) were kept under different housing conditions for 3 weeks. The Positive mood group was placed in a predictable environment rich in stimuli and with a calm human–animal interaction whereas sheep in the Negative mood group experienced a relatively barren and unpredictable environment with a harsher human–animal interaction. In the subsequent test week, 15 (8 and 7) experimental animals were exposed to four situations intended to elicit emotional reactions: separation from group members (negative valence), sham-grooming (low intensity negative), non-grooming (neutral), and grooming (positive valence). Data were tested for interactions between mood group and valence of the test situation using linear mixed models. More frequent ear-posture changes, a higher proportion of asymmetric ears, shorter inter-heartbeat intervals and higher respiration rates were observed during separation than during the other situations. Interactions between mood group and test situation showed that sheep in the Negative mood group reacted more negatively to the negative and more positively to the positive situation (proportion of asymmetric ears, inter-heartbeat interval, respiration rate). In conclusion, our findings suggest that sheep in a Positive mood reacted more weakly to an emotional situation independent of its valence, thus buffering negative events but also degrading positive events. In addition, this investigation showed the potential of using observations of ear postures and cardio-respiratory measures in sheep to assess the valence of short-term emotional states and their modulation by mood states.

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1. Introduction

The assessment of affective states in animals is an ongoing-challenge, owing to their subjectivity (Botreau et al., 2007; Désiré et al., 2002). Such affective states comprise both short-term emotional reactions and longer-term moods (Posner et al., 2005; Spruijt et al., 2001), and can be characterised by their valence and arousal (Russell, 1980). These aspects are thought to be largely independent

axes describing the state of an animal. Whereas valence describes the way an emotional stimulus or situation is perceived in respect to a negative–positive axis, arousal indicates the general state of activation in such a situation (Russell, 2003). For example a state eliciting a fearful flight reaction can be characterised by a negative valence and a high state of arousal. Arousal is not considered to necessarily have a negative effect on the animal's emotional state, it is the valence of a situation specifically that can lead to changes in the animal's welfare.

In humans, short-term affective states coincide with reactions in brain, physiology and behaviour (Désiré et al., 2006; Kreibig et al., 2007; Paul et al., 2005). These measures have indeed been used to assess both the positive and negative valence of short-term emotional states in animals as reflected by cardiac and behavioural measures (Boissy et al., 2007; Reefmann et al., 2009a). In addition, long-term Negative mood states (i.e. weeks to months in length) are known to modulate short-term reactions (Harding et al., 2004). The avoidance of negative emotional stimuli and the promotion of positive stimuli are thought to enhance animal welfare. Additionally, it seems likely that a more Negative mood may taint all emotional experiences (Grippo and Johnson, 2009) and is thus detrimental to welfare, whereas a more Positive mood may enhance welfare in that the environment is perceived more favourably in general (Mendl et al., 2010) and single negative events might be buffered (van der Harst and Spruijt, 2007). The relation between mood and short term emotional reactions may be more complicated, though. Muehlemann et al. (2011), for example, found a stronger brain reactivity of sheep in a more Negative mood towards a positive stimulus of being groomed. In spite of the importance of a possible interaction of mood and short-term emotional experiences for animal welfare, few studies have addressed this topic except for clinical states of Negative mood such as depression.

Animals' moods may differ as a result of their housing and husbandry environment (Burman et al., 2008) in response to aspects such as enrichment, predictability of husbandry procedures and quality of human–animal interactions. Environmental enrichment, including the provision of additional space, objects for manipulation, and social companions, are known to have long-term benefits for animal welfare (Gygax et al., 2007; King, 2003; Sato et al., 1993; van der Harst et al., 2003), and are thus likely to induce a Positive mood. By contrast, husbandry conditions that include cage tilting, wet bedding, foreign smell or reversed light–dark cycle have been used to induce chronic mild stress in rodents, a negative affective state used to model human depression (Negative-mood disorder; Grippo and Johnson, 2009; Willner, 1997). These clinical states may have much more serious implications for the animal than being in a bad mood, such as serious neuropharmacological and neuroanatomical changes (e.g. Dagyte et al., 2011; Kupferschmidt and Zakzanis, 2011; Wu et al., 2011). On the other hand, a non-clinical Negative mood state may be highly relevant for the assessment of emotional situations and thus for animal welfare. Unfortunately, previous research on Negative mood states in animals and humans has largely focussed on clinical depressions. Furthermore, animals housed under

unpredictable housing conditions (Doyle et al., 2011; Harding et al., 2004; Mendl et al., 2009) more often react to ambiguous stimuli as if they were negative (so-called cognitive bias), and chronic mild stress can induce reduced responsiveness to positive stimuli (Grippo, 2009). With respect to human–animal interactions in commercially housed animals, rough, aversive (e.g. shouting) and unpredictable handling can increase an animal's fear of humans (Boivin et al., 2003; Waiblinger et al., 2006).

Consequently, lack of enrichment, unpredictable management and harsh human–animal stockmanship may all shift the mood of animals towards a more negative valence, and modulate the individual animal's reaction to short-term stimuli. By contrast, there is evidence that repeated positive events can enhance an individual's mood state: animals having access to enrichment appear to be less anxious, and show a more positive anticipation of events in the near future (Spruijt et al., 2001; van der Harst et al., 2005). Behavioural and physiological studies suggest that animals prefer predictable and controllable events to unpredictable and uncontrollable ones (Bassett and Buchanan-Smith, 2007; Greiveldinger et al., 2009). They may also perceive short-term negative events as less stressful when in a Positive mood (Manteuffel, 2006). In addition, the quality of human–animal interactions can be beneficial: caretakers who groom, brush (Schmied et al., 2008), stroke, tickle (Burgdorf and Panksepp, 2001) or talk gently to animals can diminish fear reactions and ease handling (Bertenshaw and Rowlinson, 2008; Boivin et al., 2003; Hargreaves and Hutson, 1990; Waiblinger et al., 2006). Thus, enriched and predictable housing conditions with positive human–animal interactions are likely to ameliorate the animals' mood and coincide with good welfare (Bateson and Matheson, 2007; Matheson et al., 2008).

The present study investigated whether and how behavioural and physiological measures used to differentiate short-term emotional states of positive and negative valence in sheep are modulated by different mood states. Two groups of sheep were thus subjected to different housing and husbandry treatments that differed in environmental enrichment and disruption for three weeks, in order to induce a more Positive or more Negative mood state (Doyle et al., 2011). During the fourth week (test week), the animals' behavioural and physiological reactions were observed during short-term situations of presumed negative (separation), neutral (non-grooming) and positive emotional valence (grooming), in addition to a control situation with a presumed low intensity negative emotional valence (sham-grooming; Reefmann et al., 2009a). Measures that have previously been useful for differentiating the valence of emotional reactions (Boissy et al., 2011; Reefmann et al., 2009a,b) were chosen to assess the modulation of short-term emotional reactions by mood. These had included observations of ear postures, cardiac activity (inter-heartbeat interval and heart-rate variability), and respiration rate over a period of 30 s in each test situation. In line with these previous experiments, we expected the sheep to exhibit more ear-posture changes and asymmetric ear postures, and have shorter inter-heartbeat intervals, lower heart-rate variability and higher respiration rates when exposed to a negative as

opposed to a positive emotional situation regardless of their mood state. However, given that mood can modulate emotional reactions, we expected the reactions of the sheep in a Negative mood to be more severe compared to those of sheep in a Positive mood, as to reflect a more negative interpretation of their situation.

2. Material and methods

2.1. Animals

Nineteen non-gestating, non-lactating female sheep (nine 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. Since then, they had been housed together as a group at the Agroscope Reckenholz-Tänikon Research Station ART (Tänikon, Switzerland; Reefmann et al., 2009a,b,c) until the current experiment was conducted. Before the experiment, water and hay were available ad libitum and the hayrack was refilled twice daily at around 07:30 and 15:00 h. In the years preceding the current experiment, sheep were on pasture at times from spring to autumn when no experiments were being conducted. The study was approved by the Federal Veterinary Office (Switzerland) and licensed by the Cantonal Office (Frauenfeld, Thurgau, Switzerland, F2/06). After the experiment, the sheep were given into privately owned small flocks kept either as a hobby or for landscaping.

2.2. Experimental design

The experiment was conducted in April and May 2008 and lasted for four consecutive weeks. During the first three weeks, animals were housed in two groups to induce a more Positive or more Negative mood state (Positive and Negative mood group). In the fourth week, the test week, the emotional reactions of the sheep and the modification of these reactions by their mood state were assessed. In the assignment of sheep to the mood groups, we wanted to avoid any bias potentially induced by differing emotional reactivity of the two breeds and by how eager sheep were of being groomed (the positive stimulus) in general. Within the breeds, half of the sheep that freely approached the familiar experimenter (NR) to be groomed and half of the sheep that had to be actively approached by the experimenter for grooming were thus randomly assigned to the two mood groups. In each group there were also two sheep that avoided being groomed and which were used as companion animals during the tests. We ended up with a total of 15 sheep as experimental subjects.

2.3. Positive and Negative mood treatments

Ten sheep (four Swiss White Alpine and six Lacaunes; 70 ± 10 kg) were subjected to measures presumed to induce a more Positive mood. These sheep remained in their familiar housing environment, consisting of an open-front pen (58 m^2) comprising an area with deep-litter straw bedding (42 m^2) and a feeding area with a solid concrete floor. The feeding area was composed of two compartments

(5.5 m^2 and 10.5 m^2) that could be separated by an opaque wooden door. Also, this feeding area could be separated from the deep-litter area with a similar opaque wooden door. Overall floor area and space at the hayrack were 5.8 m^2 and 70 cm per sheep, respectively. Whilst feeding on hay in the morning, animals also received pellets (UFA 250 pellets, NEL 4.0 MJ/kg) as a treat. During dry weather conditions, the sheep had access to an additional outdoor exercise yard (18 m^2). To mimic a good human–animal relationship, the person feeding the animals (NR) spoke quietly or hummed without making any rough movements or loud noises, and treated the animals gently. During the period of mood induction, animals were let out on pasture together as a group for 0.5–2 h every second or third day (seven or eight times, depending on whether testing in the test week was early or late for the animal in question).

The nine sheep in the presumed Negative mood group (five Swiss White Alpine and four Lacaunes; 65 ± 11 kg) were housed in a different building nearby the Positive mood group. The pen, which was unfamiliar to them, comprised a deep-litter straw area (3.75 m × 3.90 m) in a closed building with less space per animal (1.6 m^2 per sheep and 40 cm at the hayrack) and darker lighting conditions (on average 150 lx less than in the Positive mood group as measured at three locations within the pen using a hand-held lux meter; Elvos LM-1010, Elvos GmbH, Ludwigsburg, D). These housing conditions still conformed to Swiss animal welfare legislation. The animals were fed a pure hay diet with no extra pellets. Feed delivery was irregular, and on several occasions was deliberately delayed after being prepared in front of the animals, so as to render feeding times unpredictable. To mimic a harsher human–animal relationship, the person delivering the feed (LG) made noise, shouted at the animals and rushed around the pen, pushing the sheep away or chasing them over a short distance for about one minute. Several times over the three weeks, an umbrella was unexpectedly opened in front of the animals. Sheep in this group were not given access to pasture. Instead, held by the collar, animals were taken away from their flock one-by-one and pushed or pulled along unpredictable paths for one to five minutes. Similarly to the mood group, each animal was moved 7–8 times every second or third day in order to provide physical exercise comparable to that of the Positive mood group. This was done at different times of the day, and sometimes directly before feeding time. This treatment is very similar to the one applied by Doyle et al. (2011) and some of its aspects may well occur on working farms.

In our mood treatments we varied aspects of the sheep's housing environment known to influence their welfare. For example sheep may suffer from restricted space allowance (Bøe et al., 2006; Petherick and Phillips, 2009), face more competition and changes of their feeding behaviour with a reduced feeding space to animal ratio (especially if fed hay; Bøe and Andersen, 2010), profit if provided with a variety of flooring materials (Faerevik et al., 2005), access to the outdoors and adequate lighting conditions (Caroprese, 2008) and from a good human–animal relationship (Waiblinger et al., 2006) and sheep deal more easily with predictable negative events (Greiveldinger et al., 2007).

To disentangle the effect of the valence of our experimental stimuli from a potential effect of novelty, animals of both groups were familiarised with the situations, which would be used to assess their affective reactions in the test week during the three weeks of mood induction. Every second to third day, the experimenter (NR) groomed each sheep without using any kind of fixation. She waited for the sheep to approach or, if an animal did not approach her after 30 s, she slowly approached the sheep in its corresponding home pen. Once body contact was established without the sheep moving away, she groomed the preferred body area of the animal (ventral or lateral neck, breast or belly, as observed in preliminary investigations) with approximately 0.5–1 stroke/s. Each sheep had been groomed 8–9 times before the test week. In addition to the grooming, each animal was separated from group members to familiarise but not habituate it to being alone. In the two weeks preceding the test week, an experimenter separated each sheep from the flock once a week (week one, NR, separation: 45 s; week two, LG, 90 s).

2.4. Test week: assessment of emotional reactions and their modulation by mood

During the test week, sheep were subjected to four situations likely to elicit different emotional reactions ranging from negative to positive: separation as a negative emotional situation, sham-grooming as a situation of low intensity negative emotional valence, non-grooming as a neutral situation, and grooming as a positive situation (cp. Reefmann et al., 2009a). Sham-grooming consisted of counting down the start and the end of a short period when sheep could expect to be groomed (see below; counting was necessary for the measurements presented in Muehlemann et al., 2011) but sheep were not groomed and not otherwise interacted with. Thus, sham-grooming served as a direct control situation for grooming. Grooming of the sheep was considered positive since the sheep selected for the experiment either approached the familiar human experimenter of their own volition to be groomed or did not walk away during grooming. As described above, the sheep were not tied in any way, and some directed various attention-seeking behaviours at the experimenter (e.g. establishing body contact and nibbling clothes). The non-grooming situation was assumed to be more or less neutral whereas sham-grooming was considered a low intensity negative situation (frustration-like). The separation effect was always tested last, in order to avoid a carry-over effect of negative valence and arousal to the other more positive and presumably more subtle situations.

The experiment was conducted daily between 08:00 and 15:00 h over a one-week period and lasted up to 30 min per sheep. The testing schedule was balanced for mood group and time of day, and animals from both groups were tested on each day. All animals of one of the two mood treatments were moved to the feeding area of the open-front pen. The experimental animal was then moved to the small compartment of the feeding area for testing together with one companion sheep whilst the other animals of the group stayed in the larger compartment. For the presumably positive emotional situation of being groomed,

sheep were groomed ten times for 45 s each (as described above). 30 s before the first and after the last grooming as well as 60 s between the grooming stimuli were considered non-grooming periods representing a situation of neutral emotional valence. The eighth 45 s grooming period was additionally replaced by omitting the grooming as a control situation ('sham-grooming'). After the final non-grooming period, the companion sheep was released into the larger section of the feeding area to join the other sheep of its mood group. The female experimenter then left the compartment, and the experimental sheep was left alone with a stranger (TM) for 60 s, before being visually separated from group members and humans for 45 s (negative test situation) but remaining in auditory and olfactory contact with their group members.

During the test session, the experimental sheep was fitted with devices to measure mean inter-heartbeat interval (inverse heart rate), heart-rate variability (RMSSD: root mean square successive difference of inter-heartbeat intervals), respiration rate (for details see Reefmann et al., 2009c) and functional near-infrared spectroscopy (Muehlemann et al., 2011). The sheep had been habituated to wearing the equipment in several previous experiments, and were familiarised with it at least another three times before the test week. The day before the experiment, sheep were depilated on the relevant areas of their body (patches on the rump for electrocardiogram electrodes). A camera was installed above the small compartment of the feeding area in order to observe the ear postures of the sheep during each situation, and relayed images were recorded with a videotape recorder (Time Lapse Recorder AG-6040 E, Panasonic).

2.5. Data analysis and statistics

Ear postures of the sheep during the experimental situations were analysed according to the definitions of Reefmann et al. (2009b). Briefly, the animals' total number of ear-posture changes (i.e. from forward, to axial, to backwards) per 30 s' observation time was noted, as were the proportion of asymmetric ear postures (i.e. right and left ear in a different posture), and the proportion of left-lateralised ear postures (i.e. number of times the left ear was further forward than the right ear, divided by the sum of this number plus the number of times the right ear was further forward than the left ear). Physiological measures were analysed as described in Reefmann et al. (2009c). One data point was calculated per experimental test situation and per sheep for each behavioural and physiological variable. This was done using the 30 s in the middle of each of the following situations: the seventh grooming period, the following non-grooming period, the subsequent sham-grooming period, and the separation when the sheep was left alone. For one animal of the Positive mood group, no values were available during the seventh grooming period due to poor data quality, so values from the very first grooming period were used. With another animal in the Positive mood group, the respiration belt failed to store data, so all data from a repeated experimental trial were used for analysis. Where available, this data was not clearly different from the original trial, which could be expected

as all animals were familiarised with the experimental stimuli.

Each behavioural and physiological variable was modelled separately as a response variable using linear mixed models (Pinheiro and Bates, 2000) in R (version 2.12.2; R Development Core Team, 2011). A random effect for individual sheep was included in all models to account for the repeated testing of individuals (once in every of the four test situations).

Even though mood induction was simultaneously induced in all sheep of a given treatment group and it could be argued that our sample size is thus only two, we used the singly tested sheep as our unit of replication. It is very unlikely that sheep within the mood groups had affected each other in a way which did not alter mood but consistently affected the response to our stimuli.

Explanatory variables were test situation (within-subject: grooming, non-grooming, sham-grooming and separation, either coded as equidistant values 1, 2, 3, 4 on a continuous scale or as an ordered factor) and mood group (between-subject: Negative and Positive, factor with two levels). Statistical model assumptions of normal distribution and homoscedasticity of the model errors were checked by a graphical analysis of the residuals based on the model that included test situation as an ordered factor and an interaction between test situation and mood group (the maximum model given our experimental design). To satisfy assumptions, the proportion of left-lateralised ear postures was logit transformed and all physiological variables were log-transformed. Owing to large differences in the variance of data between the test situations, a term accounting for this heteroscedasticity was included for all ear postures and the mean inter-heartbeat interval.

We chose the final model to be presented based on information theory using Akaike's information criterion (IT-AIC; Garamszegi, 2011; Symonds and Moussalli, 2011; Burnham et al., 2011) because classical step-wise backwards methods based on null hypothesis significance testing (NHST) face serious drawbacks in respect to bias, multiple testing and model selection uncertainty (Whittingham et al., 2006; Lukacs et al., 2007). The IT-AIC approach chooses an optimal model in the sense that bias is minimised with a minimum of fixed effects. Instead of using the NHST approach to calculate the probability that the observed data could have resulted from a process specified by the null hypotheses (that may be unlikely to begin with, Burnham et al., 2011), IT-AIC provides probabilities for each of several concurrent models given the data (Symonds and Moussalli, 2011; Burnham et al., 2011). The models can additionally be compared based on evidence-ratios, which quantify the relative probability of different models (Symonds and Moussalli, 2011; Burnham et al., 2011). We used functions in the package AICcmodavg in the process of the analysis (Mazerolle, 2011).

In a first step, we checked whether the pattern across testing situations (from negative to positive) was close to linear or not. If not, this could either be due to these patterns being non-linear, u- or inversed u-shaped or due to our presumed sequence from negative to positive being incorrect. To check linearity, we compared the model including the interaction between testing situation and

mood group, in which testing situation was coded as an ordered factor, with the same model in which testing situation was coded as equidistant values based on the models' AIC_c values (Table 1, "Coding of valence").

In a second step, we compared several models with the coding of testing situation that was found to be appropriate in the first step. We used AIC_c values to compare the model with the interaction between test situation and mood group with the main effects model, with the model including mood group only, the model including test situation only and the model including a constant (intercept) only (Burnham et al., 2011; Dochtermann and Jenkins, 2011). This last model corresponds to the null hypothesis that neither test situation nor mood group had an influence and that the responses randomly vary around a general mean (Table 1, "Choice of model complexity").

Whilst we followed the advice to choose the simpler model if models are nested and have similar AIC values (Richards et al., 2011) in our first step, we did chose the more complex models in the second step if AIC values were similar and model probabilities of the more complex models were considerable, too. We did so because we were investigating subtle effects and at the time being we wanted to avoid false negatives. Nevertheless, this choice in how the approach was applied needs to be considered in discussing the results.

As is considered good practice by e.g. Mundry (2011), we show and interpret the estimated models based on the model parameters and their variability, which we show in the form of model estimates including 95% confidence intervals plotted onto the raw data presented in boxplots (Fig. 1).

3. Results

3.1. Model selection

All response variables except the number of ear posture changes showed a good to very good support of a linear pattern in response to our equidistant coding of the test situation (94–96%, step one; Table 1, "Coding of valence", bold numbers). In the case of the respiration rate, the model probability still reached 48%, which indicates a 50:50 chance of the simpler model being more correct. Thus, we retained the test situation as an ordered factor for the ear posture changes whereas all other response variables were modelled using the equidistant coding of test situation.

In the second step, we chose models that had at least a one in six chance of being the best models (Table 1, "Choice of model complexity", bold numbers with an !). The AIC_c values of these models also differed by less than 3 from the AIC_c value of the best model so they can virtually be considered equivalent (Symonds and Moussalli, 2011; Burnham et al., 2011). This resulted in the main effects model for the number of ear postures and the model including the interaction for the proportion of asymmetric ear postures, the proportion of left-lateralised ear postures, the mean inter-heartbeat interval and the respiration rate. For the RMSSD, the two models with the single main effect reached similar model probabilities. As

Table 1

Measures used in model selection of variables valence (V) and mood (M) based on AIC_c values. See text for further explanations.

	Measures[a]	Coding of valence		Choice of model complexity				
		$V \times M$	$V \times M$	$V \times M$	$V + M$	M	V	Intercept
df linear[b]			3	3	2	1	1	0
df ordered[b]		7		7	4	1	3	0
Number of ear-posture changes	AIC_C	314.10	319.48	314.10	310.38	355.79	307.54	354.74
$ER_0 = 19.00$	Δ_i		5.39	6.56	2.84	48.25		47.20
	w_i	**0.94**	0.06	0.03	**0.19!**	0.00	**0.78**	0.00
	ER		15.67	26.00	4.10	>156.00		>156.00
Proportion of asymmetric ear postures	AIC_C	−34.08	−42.08	−42.08	−39.72	−16.51	−42.31	−19.03
$ER_0 > 41.00$	Δ_i	8.01		0.23	2.59	25.80		23.28
	w_i	0.02	**0.98**	**0.41!**	0.13	0.00	**0.46**	0.00
	ER	49.00		1.12	3.54	>92.00		>92.00
Proportion of left-lateralised ear postures	AIC_C	148.36	142.15	142.15	142.62	141.37	141.22	141.32
$ER_0 = 0.67$	Δ_i	6.21		0.93	1.40	0.15		0.09
	w_i	0.04	**0.96**	**0.16!**	0.12	**0.23**	**0.25**	**0.24**
	ER	24.00		1.56	2.08	1.08		1.04
Mean inter-heartbeat interval	AIC_C	−96.57	−99.43	−99.43	−98.86	−93.02	−101.52	−95.57
$ER_0 = 7.00$	Δ_i	2.85		2.09	2.66	8.50		5.94
	w_i	0.19	**0.81**	**0.21!**	0.16	0.01	**0.59**	0.03
	ER	4.26		2.81	3.69	59.00		19.67
RMSSD	AIC_C	103.25	96.89	96.89	96.01	93.63	93.68	91.38
	Δ_i	6.36		5.51	4.63	2.25	2.29	
	w_i	0.04	**0.96**	0.04	0.05	**0.18**	**0.18**	**0.55!**
	ER	24.00		13.75	11.00	3.06	3.06	
Respiration rate	AIC_C	17.98	18.13	18.13	17.83	55.61	15.52	53.39
$ER_0 > 17.00$	Δ_i		0.16	2.61	2.31	40.09		37.86
	w_i	0.52	**0.48**	**0.17!**	0.20	0.00	**0.63**	0.00
	ER		1.08	3.71	3.15	>126.00		>126.00

[a] Measures used in AIC-based model selection. AIC_C: AIC-value corrected for small sample sizes; Δ_i: differences in AIC_C in comparison to the optimal model (having the lowest AIC value) within the set of models; w_i: Akaike weight which can be interpreted as the probability of the given model within the presented set; ER: evidence ratio, the optimal model is ER times more likely than the given model.

[b] df: degrees of freedom used by the fixed effects in the model (all other estimated parameters, i.e. the intercept, the random effect of sheep, the variability of the error and variance estimates in the heteroscedastic models are included in all these models). Empty fields: $\Delta_i = 0$ and ER = 1 in the optimal models. !: final model reported. ER_0: evidence ratio between the chosen model and the null model (including the intercept only).

neither the combined main effects model nor the model with interaction seemed to be a good description of the observed patterns, this result seems to be spurious and we chose the null model that had clearly reached the highest model probability (Table 1).

We also reported the evidence ratio of the chosen model in comparison to the null model (including a constant only) for those response variables for which a model other than the null model was chosen (ER_0 in Table 1). In all instances except for the proportion of left-lateralised ears, this evidence ratio was high (>7) and thus clearly discounting the null model. The evidence ratio for the proportion of left-lateralised ears was 0.67, and thus needs to be considered relatively weak evidence in comparison to the null model.

3.2. Effects of valence and modulation by mood

The number of ear-posture changes was high during separation, low during grooming and on an intermediate level during sham-grooming and non-grooming. Though mood was retained as a main effect in this model, there was no discernible shift of the reaction between the two mood groups (Fig. 1a).

The proportion of asymmetric ears decreased from the negative to the positive situation. Sheep in the Negative mood group showed a steeper decrease in their reaction from the negative to the positive test situations which is related to a wider range in their reactions, i.e. sheep of the Negative mood group reacted more (negatively) towards the negative and more (positively) towards the positive test situation compared to the sheep in the Positive mood group (interaction, Fig. 1b). The same kind of pattern was found for the respiration rate (Fig. 1f) and with an inverse sign for the inter-heartbeat interval (Fig. 1d).

The proportion of left-lateralised ears was similar for animals in both mood groups in the negative test situation but decreased towards the more positive situations in the animals of the Negative mood group, whereas it increased

Fig. 1. Behavioural (a)–(c) and physiological responses (d)–(f); RMSSD = root mean square of successive differences of inter-heartbeat intervals) of sheep to affective stimulation. Short-term emotional situations were separation from group members (S; negative valence), sham-grooming (h; control, low intensity negative valence), non-grooming (n; neutral), and grooming (G; positive valence). All measures were assessed over periods of 30 s for sheep of a Negative and Positive mood group, characterised by different housing and husbandry procedures. Raw data are given as boxplots with median, inter-quartile and absolute data range, in addition to estimated model means (thick line) and 95% confidence intervals (thin lines). N = 15 sheep in all boxes except in (c), where sample sizes were 7, 5, 5, 0, 8, 5, 7, 3 (from left to right) due to some sheep not showing any asymmetric ear-postures.
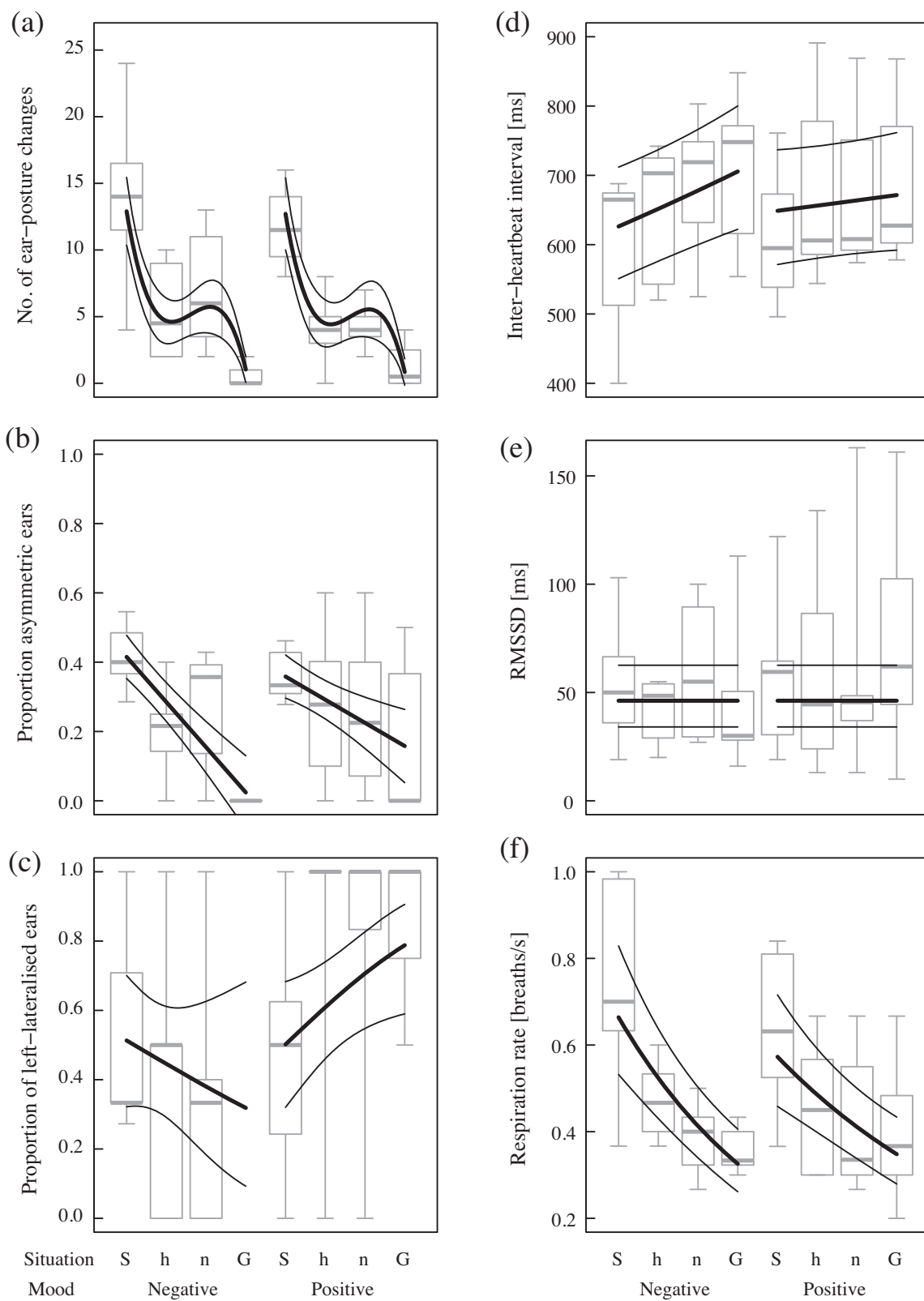
Fig. 1.

in the animals of the Positive mood group (interaction, Fig. 1c).

There was no discernible effect of test situation nor mood group on RMSSD (Fig. 1e).

4. Discussion

Both behavioural and physiological measurements (except RMSSD) were useful for differentiating positive and negative short-term emotional responses of the sheep as supported by the highest model probability reached by the single effect model including test situation as the predictor (Table 1, "Choice of model complexity"). In the negative emotional test situation (separation from group members), the number of ear-posture changes, the proportion of asymmetric ear postures, and in particular the respiration rate were highest, whilst the mean inter-heartbeat intervals were shorter than in the situation of positive emotional valence, when sheep were being groomed by a familiar person. Thus, we successfully replicated data from previous experiments assessing short-term emotional reactions in different situations (Boissy et al., 2011; Reefmann et al., 2009a,b,c). These data are also in accordance with data from humans and animals on increased mean heart rate (i.e. inverse mean inter-heartbeat interval) and respiration rate during negative emotion (Cabanac and Cabanac, 2000; da Costa et al., 2004). Effects of positive emotions on mean heart rate are less straightforward, whereas Matsunaga et al. (2009) did not find changes in heart rate in humans watching a positive movie, Zebunke et al. (2011) found an increase of heart rate in pigs during a learning situation judged to be positive. The latter finding seemingly contrasts our results but animals needed to be active in that task whereas they were passive in our positive treatment (grooming). Contrary to our previous observations of sheep during separation and whilst being groomed (Reefmann et al., 2009a), RMSSD did not react differentially in response to the test situations in this current study. The observed values were similar to those in the intermediate situation tested in Reefmann et al. (2009a) but did not reach values as low as during separation, nor as high as the values during grooming in the earlier study. This can potentially be explained by a lower responsiveness of the parasympathetic system due to habituation in our sheep to these specific test situations.

It could be argued that the changes observed in our outcome variables were not caused by the valence of the test situations but by the arousal induced by these situations. It is likely that the separation caused a state of high arousal by activating a fight–flight response in contrast to being groomed, which is generally assumed to cause a relaxed state. In a previous experiment (Reefmann et al., 2009b,c), we used a paradigm in which a feed expectation was either disappointed or surpassed to induce situations of different emotional valence. In this case, one would assume that the disappointment would lead to a negative state with a reduction in arousal (frustration) whereas a surpassed feed expectation is likely to result in an agitated positive state. That is in the earlier study, the positive situation was arousing and the negative situation was calming whereas in the current experiment, the negative situation was arousing and positive was calming. In spite of these differences between experiments, we found a decrease in the number of ear-posture changes, in the proportion of asymmetric ears, and in heart rate from the negative to the positive test situation. This leads us to conclude that the observed reactions are mainly caused by the valence of the test stimuli rather than the arousal caused in these situations. Nevertheless, more negative and positive stimuli and situations need to be tested to make a general case in respect to these indicator variables for valence.

The influence of our mood treatment was less straightforward than the influence of the (valence of the) test situations. None of the models including mood was observed to be the optimal model, but in four out of six outcomes the model including the interaction between test situation and mood group had a model probability higher than one out of six. This is not negligible and indicates that emotional reactions in sheep were modulated by mood, although the investigated effects are subtle. The fact that the sheep in our two treatment groups for mood showed a differential reaction towards the experimental stimuli differing in valence indicates that our simultaneous manipulation of several aspects of the animals' housing and husbandry environment (i.e. enrichment, predictability of husbandry procedures and human–animal interaction) was effective. In a future experiment, it would be advantageous to have an independent measure of the differential moods induced in the experimental subjects, i.e. based on a cognitive bias test (Doyle et al., 2011). Nevertheless, based on previous experiments with a variety of species and specifically sheep (Doyle et al., 2011), it is likely that our sheep were indeed in a relatively more Negative and Positive mood state. Also, our results are consistent with that notion.

In respect to our mood groups, we can differentiate three reaction patterns. First, sheep in the more Positive mood reacted less extreme to both the separation (negative stimulus) and being groomed (positive stimulus) regarding the proportion of asymmetric ears, the inter-heartbeat interval and the respiration rate (the response curves of the two mood groups cross). This is in line with theories on the buffering of negative events (such as separation) when in a Positive mood (Manteuffel, 2006), and theories of more pronounced stress reactions in already stressed or even depressed individuals (Grippo and Johnson, 2009), i.e. those in a Negative mood. Contrary to our expectations, the sheep in the more Positive mood also reacted less strongly in response to the positive stimulus (grooming). We cannot currently differentiate whether this is the result of a stabilising effect of a more Positive mood or due to a generally increased excitability in a more Negative mood. Interestingly, a similar pattern was also found in cerebral tissue oxygenation related to brain activity (Villringer and Chance, 1997): our sheep in the presumably Negative mood group showed a stronger response to grooming than those in the Positive mood group (Muehlemann et al., 2011). Animals in a Positive mood that are frequently exposed to positive stimuli may become habituated to short-term positive experiences. This may then lead to an adjustment of their expectations, and would explain the weaker

behavioural and physiological reaction to familiar positive stimuli in the Positive mood group. By contrast, animals in a Negative mood may have different expectations, and might therefore find a familiar positive stimulus to be particularly pleasurable. The changes in inter-heartbeat intervals may also correspond to an increased sympathetic drive during depression or depression-like states in humans and animals (Grippo et al., 2006; Grippo, 2009).

Second, the proportion of left-lateralised ears was similar in both treatment groups for mood in the negative situation but these reactions weakened in the more Negative mood group and intensified in the more Positive mood group when moving from the negative towards the positive stimuli. Previous studies on emotional processing in the brain suggest that laterality of emotional valence is related to lateralised behaviour patterns in dogs and sheep (Quaranta et al., 2007; Reefmann et al., 2009b), and may explain the different patterns for our two mood groups. The mechanisms, however, remain to be investigated. In addition, it has to be noted, that the support of this modulation was relatively weak in that a strong candidate model of the proportion of lateralised ears was the null model. Third, some of the reactions towards emotional stimuli did not vary with mood to an extent that was detectable: the sheep's ear-posture changes did not appear to be modulated by mood and thus seem to be less sensitive in picking up mood.

Given the linear reaction pattern in the observed outcomes, we seemed to have assigned the correct sequence of valence to the test situations: sham grooming is seemingly more negative for the sheep than the intervals between grooming bouts (non-grooming). Thus, the expectation of an event that does not occur seemed to be more negative than an interruption of a pleasant stimulus. Also, the sheep may have quickly learned during training that grooming is resumed after a short time. The intervals between grooming bouts had induced a similar amount of ear-posture changes as did sham grooming. Seemingly, the level of necessary alertness was similar in those two situations in our sheep.

In this study, the proportion of asymmetric ear postures, the inter-heartbeat interval and the respiration rate appeared to be the most useful criteria for assessing the modulation of animals' short-term emotional responses by a mood state presumably induced by differential housing and husbandry conditions over a three-week period. This is in line with previous studies showing that in rats or prairie voles, four weeks of differential handling is sufficient to induce differences in cognitive, behavioural and physiological responses to emotional events (Grippo, 2009; Mendl et al., 2009).

Given that the presumed mood of the animals was influenced by differential housing conditions, future experiments will need to disentangle which of the various manipulations – i.e. physical enrichment, unpredictability of husbandry procedures, or quality of human–animal interaction – had the greatest effect on mood. Those results can then be used to improve the relevant aspect of the animals' housing environment with the aim to induce Positive mood states in the animals, thereby improving their quality of life (Boissy et al., 2007).

5. Conclusion

Short-term positive and negative emotional states in sheep were clearly differentiable by observing ear postures, inter-heartbeat intervals and respiration rate. In particular, the proportion of asymmetric ears, the inter-heartbeat interval and respiration rate were influenced by mood group. Consequently, these measures appear to be suitable for detecting a modulation of short-term emotional responses by underlying long-term affective states in sheep. A more Positive mood appeared to buffer the stress reaction to a negative event, e.g. separation from group members. Interestingly, sheep in a Negative mood showed an enhanced reactivity to positive situations, indicating that they may have experienced a positive short-term situation as more pleasurable than the sheep in a Positive mood. Hence, data presented here provide a first step in the use of behavioural and physiological measures to assess the effect of long-term Positive affective states (i.e. mood) on short-term emotional reactions, with the aim of improving the assessment of subjective states in animals, and thus enhancing animal welfare.

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