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Welfare by the ear: comparing relative durations and frequencies of ear postures by using an automated tracking system in sheep

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Abstract

Given the increased interest in animal emotional reactions for assessing welfare, indicators for such reactions are sought. Ear postures and movements have been found to be promising indicators of emotional states in sheep and other animals. The manual recording of ear postures, however, is very time consuming and possibly prone to a degree of inaccuracy due to the subtle and fast nature of ear movements that have to be identified. Therefore, a number of previous studies have analysed the frequency of certain ear postures relative to all ear posture changes rather than measuring the relative duration spent with different ear postures. Here, we present an automated, continuous tracking system that keeps track of small and lightweight marker balls attached to the head and ears of sheep. We measured ear postures and movements when the animals were confronted with three physical stimuli thought to differ in valence (from negative to intermediate to positive). We then compared new ear-posture definitions reflecting the real time spent with certain ear postures during stimulation with previous definitions used for video-based analyses that assessed ear-posture changes in relation to the total number of observed ear postures. In the analysis, we correlated new and previous measures both between and within experimental stimuli using residuals from mixed-effects models. We found that the new and previous definitions of ear postures and movements correlated highly. Given these high correlations and the discussed theoretical and practical advantages of the automated tracking, the new recording system can be recommended highly for assessing reactions in animals that may indicate emotional states.

Keywords: *animal welfare, automated tracking, ear posture, emotion, sheep, video analysis*

Introduction

The enhancement of positive as well as the reduction of negative emotions is thought to improve animal welfare (Boissy *et al* 2007). However, the assessment of short-term emotional states is difficult (Mendl *et al* 2009), and the development of objective indicators reflecting emotional states is therefore necessary. Non-invasive methods are especially valuable because their use only minimally disturbs the behaviour of the tested animals.

Ear postures and movements have been used as subtle indicators of animal emotion (dairy cows: Schmied *et al* 2008; pigs: Reimert *et al* 2013; sheep: Reefmann *et al* 2009a,b; Boissy *et al* 2011). All these studies included the definition of a set of specific ear postures, for example, measuring how far forward or backward both ears were positioned relative to the longitudinal axis of the head. For the actual measurements of the ear postures, the researchers used video observations. Video recordings have the advantage of being able to depict a general view of a situation, record several behaviours simultaneously, be relatively simple in handling, and be used widely. On the other hand, video analyses have a number of disadvantages, the main one being the very time consuming scoring after the experiments have been conducted, specifically when the video shots need to be slowed down for scoring. Also, a suitable camera array may be difficult to be determined *a priori*, and several cameras may be required to ensure that all ear movements are detectable for scoring from the video track (Tami & Gallagher 2009). This, in turn, causes additional effort in scoring pictures from each of the cameras. In the studies by Boissy *et al* (2011) and Greiveldinger *et al* (2009), for example, four cameras were used simultaneously. Another disadvantage of video analyses is the potential subjectivity of the experimenter in analysing the video data, specifically when small and fast movements of the ears are to be scored. This makes an inter-observer test of reliability advisable (Schmied *et al* 2008; Verbeek *et al* 2012) and implies an additional effort.

Therefore, in different fields of research, less time-intensive methods for analysing behaviours or movements have been examined. One of the ideas for making the analyses less time intensive was the segmentation of moving targets from the background and the tracking of the segmented individual objects afterwards (Courtney 1997; Gong & Caldas 2011). A similar method was implemented for recording animal behaviour, for example, in laboratory animals by Crispim *et al* (2012). Nevertheless, these methods were all based on video recordings and thus needed intensive handling of the large amount of image data. We were therefore looking for a specific system for tracking ear positions independent of video analysis. We found it in an automated tracking system used mostly for capturing human movements, but similar systems were used, for example, for studying lameness in horses (Pfau *et al* 2007). This new system combines a very precise data acquisition with a small effort in subsequent data analysis. Due to the automatic processing of the input from all involved infrared cameras, the resulting data set can be analysed directly without the additional step of generating digitised data as it is required for video observations.

Classically, one might consider a comparison of the new automated system with the former video-based system by using the same definitions of a set of ear postures. Given the very high physical accuracy of the automated system, it is hard to argue that the video analysis by a human observer should be the 'gold standard'. If differences resulted from the comparison, the only allowable conclusion would be that the automated system was more accurate than the human observer. Thus, this classic comparison seems trivial. However, it is of interest to check whether the recording of (relative) durations of specific ear postures, which are easily accessible given the automated system, yields results comparable with (relative) frequency measures, which have previously been used in video observations. If specific postures are indeed indicators of emotional reactions, it is likely that the duration for which the postures were shown is more informative, because different ear postures are likely to occur for a varying amount of time every time the posture is taken up, than the frequency with which the postures were reached.

In the current study, ear movement data recorded in the experiments by Vögeli *et al* (2014) with an automated tracking system were used. These experiments included the application of three types of stimuli varying in valence to evoke a broad spectrum of possible ear positions in sheep. The continuous data flow from the automated system allowed the assessment of the relative duration of the ear postures (new definition) and the frequency of certain positions relative to all observed ear posture changes, which was the definition used in previous video-based analyses (Reefmann *et al* 2009a,b). We compared ear posture and movement data between both definitions to investigate whether the new automated approach provides higher accuracy and requires less effort than video analyses and whether it can be recommended for further studies using ear movements and postures to assess emotional states in animals.

Materials and methods

Study animals

Twenty-four, focal, non-lactating and non-reproducing female Lacaune sheep were tested at an age of 14 to 17 months in April 2012. They were housed in groups of 14 and 15 animals, with 12 focal sheep per group. One group of sheep was living in a stimulus-poor and unpredictable housing environment, and the other group was living in a stimulus-rich and predictable environment (Vögeli *et al* 2014). This project was assessed by the Swiss National Science Foundation, and the necessary authorisation by the cantonal authorities was available (authorisation for the conduct of animal experiments, canton Thurgau No F6/10 and F4/11) ensuring adequate attention to animal welfare in conducting the experiment.

Experimental design

Ear movements were measured when sheep were experimentally confronted with three different physical stimuli thought to vary in their valence and described in detail by Vögeli *et al* (2014). Here, we briefly describe the aspects important for the current comparison.

Experimental testing was performed in an indoor test pen (2.5 × 2.0 m; length × width) close to the two housing environments but not allowing visual contact with the other sheep. The sheep were tested singly in the presence of a human experimenter and could move freely inside the test pen. Each test with a given stimulus lasted for about 30 min, and each sheep was tested with one of the three stimuli at the same time of day on three consecutive days. The order of the stimuli was balanced across sheep. Testing took place at the same time of day to control for any potential daily periodicity in the sheep's reactions. On each day, six sheep were tested, three sheep from one group in the morning and three from the other group in the afternoon. Morning and afternoon test sessions were balanced across housing groups to exclude any potential bias caused by daytime. For all 24 focal sheep, four blocks of three days each were therefore needed, totalling 12 days.

Applied stimuli ranged from presumably negative to intermediate to positive. All stimuli were applied by an automated mechanical device fixed at the upper breast of the sheep by using a belt system (designed for dogs; Ruffwear Web Master Harness, Bend, Oregon, USA). The negative stimulus was pricking without injury, where a total of four metal pins (3.5 cm, two per side of the device) moved against each other and pricked the sheep in consequence (one movement cycle lasted 15 s). The intermediate stimulus was a slight pressure of a metal plate (5.5 × 6.5 cm) against the body of the sheep. The positive stimulus was a kind of massaging effect that was produced by a constant forward-backward movement (1.2 s per motion sequence) of a metal plate to which four wooden hemispheres (2.3 cm in diameter) were attached. This massage simulated grooming which was found previously to be a positive stimulus for sheep (Reefmann *et al* 2009a,b). On a given day and for a given sheep, one of the stimuli was applied twelve times. The stimulation periods lasted 45 s and

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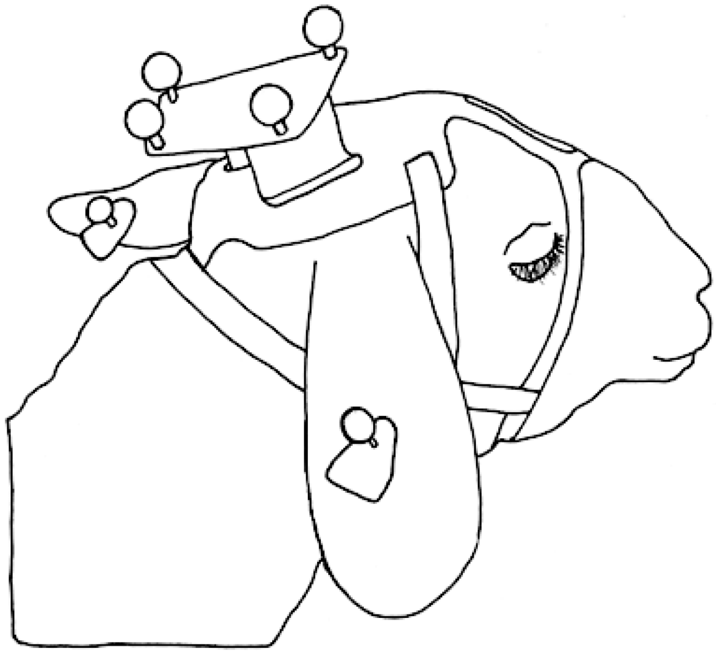
Figure 1

Targets on the head of a sheep to be tracked by an automated tracking system consisting of one single reflective marker ball on each ear and a target composed of four marker balls in a fixed arrangement attached on top of the head.



formed the basis of the current evaluation. Pauses between the single stimulations varied randomly between 55 and 65 s. To avoid an emotional reaction to the novelty of the stimuli instead of a reaction to the presumed valence, a total of 16 habituation trials were conducted before the experiments (Vögeli *et al* 2014). These trials also included the habituation of the animals to all measurement equipment described below. This habituation allowed us to assume that the measured behaviours were indeed caused by the different types of stimuli and their valence.

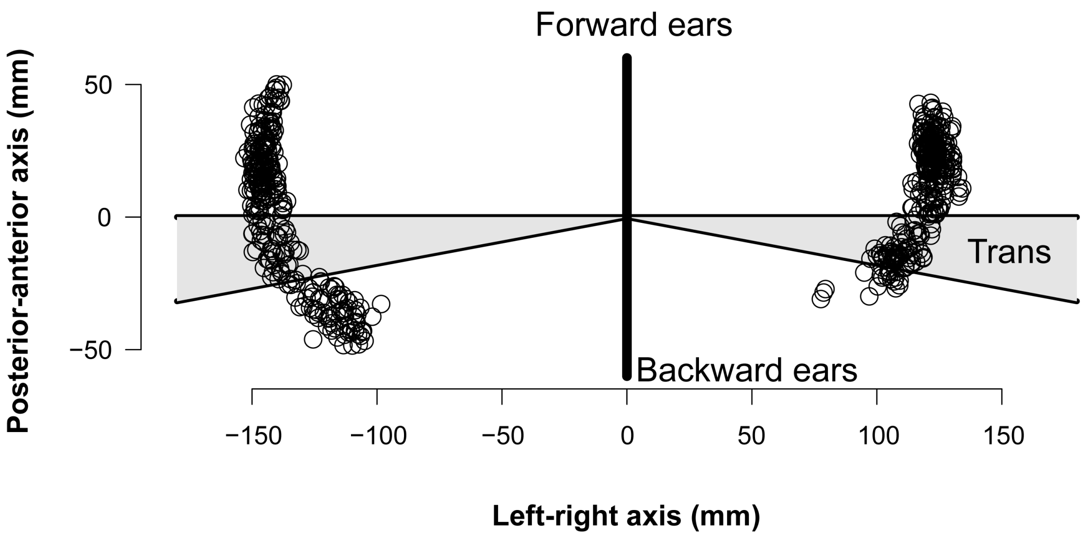
Measurement method and definitions of ear postures and movements

Ear postures and movements were recorded by an automated tracking system (Trackpack4, Advanced Realtime Tracking ART System, Weilheim, Germany) using reflective marker balls tracked by four infra-red cameras in 3D. The cameras (Trackpack, 3.5 mm focal lens, IR flash 850 nm) were placed in the four top corners of the test pen and connected with a controller (Trackpack) calculating the positions of the marker balls. These positions were then transmitted to a remote PC. A single marker ball (so-called 3D target; 12 mm in diameter, 2 g) was placed on the back side of each ear ('ear target') and fixated by a screw onto the ear mark one day before testing. Each ball could be localised in absolute 3D space. In addition, a head target (so-called 6D target) was used. It was composed of four marker balls (each ball: 14 mm in diameter, 2.6 g; whole target: 142 g) arranged in a specific configuration, which allowed not only the absolute localisation of the head target in 3D space but also its orientation, that is, its roll (sidewise tilting of the head), pitch (inclination from head to snout), and yawn (sidewise turning of the head) angles. These angles were necessary to calculate the relative positions of the ear targets in relation to the head target, because the ear targets were localised in absolute space. The head target was placed on the head of the animal in the centre between the two ears and fixated by a halter (Figure 1). Neither the head target nor the halter touched the ears. Nevertheless, animals were habituated to wearing the halter and the target as mentioned above. In fact, sheep showed no obvious reaction to the single ear targets and only slight defensive behaviour (such as head shaking and evasive movements) in response to the halter and head target at the very beginning of the habituation phase. These reactions rapidly disappeared.

Furthermore, any possible indirect effect on ear positions and movements was accounted for by the within-subject design of the experiment, which measured relative shifts between different stimuli rather than absolute values (see *Statistical analysis*). The system recorded the positions of the targets at 6 Hz. Using the co-ordinates of the ear and head targets as well as the orientation angles from the head target, we programmed a software in R (R Core Team 2013). The software first calculated the positions of the ear targets in a 3D co-ordinate space relative to the head (Figure 2) allowing for the fact that the actual centre of the head, that is, the point in the middle between the ears, was shifted 7 cm downwards and 3.5 cm towards the back relative to the centre of the head target. This shift was caused by the fixation of the head target on top of the head. In a second step, the ear positions were translated into horizontal and vertical angles describing how far forward/backward and up/down the ears were positioned.

In earlier studies by our group on ear movements in sheep (Reefmann *et al* 2009a,b), several ear postures were distin-

Figure 2



Example visualisation of the ear positions of a sheep as projected in the frontal plane, that is, as seen from directly above the head. Shown is a random sample of 300 positions from a sheep tracked for 20 min at a frequency of 6 Hz. The vertical line parallel to the Y-axis corresponds to the longitudinal axis of the head and the section of the sagittal plane. The horizontal line parallel to the X-axis corresponds to the left-right axis and the section of the transverse plane. Definitions of ear postures as used in the current study are indicated by the different sectors. The critical angle of the front sector (forward ears) corresponds to the transverse plane, and the critical angle of the rear sector (backward ears) is set 10° posterior to the transverse plane. Grey sector: transverse ears, indicated as 'Trans'.

guished and defined: the forward and backward positions of the ears were categorised by being more forward or backward, respectively, than 30° from the transverse plane, in effect splitting the 180° available for ear positions on each side of the head into three equally sized sectors. Given our more quantitative data from the automated tracking system (example in Figure 2), we realised that our previous definitions should probably have used the most forward and backward thirds of the possible ear positions instead of splitting the geometrically available space into thirds. Hence, we set the critical angles to the transverse plane itself (front critical angle) and to 10° backward from the transverse plane (rear critical angle) for all definitions of ear postures in the present study (Figure 2). We defined the following ear postures based on these critical angles: (a) forward ears (both ears being positioned further forward than the front critical angle); (b) backward ears (both ears being positioned further backward than the rear critical angle); (c) transverse ears (both ears between the front and rear critical angles); and (d) left ear forward (the left ear positioned more forward than the right ear). In addition, we calculated the total number of ear movements, that is, the activity of the ears (e).

To compare the new definitions based on the continuous, automated tracking of ear positions with the definitions previously used for video-based analyses (Reefmann *et al* 2009a,b), ear postures needed to be calculated based on the two sets of definitions. For both sets, the same original angular data of ear positions recorded by the automated tracking system were used.

New definitions based on relative durations

The proportion of time was calculated in which ears were in the forward, backward, and transverse postures (definitions [a]–[c] above). Left ear forward (d) was defined as the proportion of time that the left ear was more than 5° more forward than the right ear relative to the time when the angles between the left and the right ear were more than 5°. Ear movements (e) were estimated by the total sum of the changes in horizontal angles of both ears.

Previous definitions based on relative frequencies as used for video-based analyses

Reefmann *et al* (2009a,b) noted whenever one of the ears crossed the critical angles (as defined above). We therefore aggregated all consecutive data points as long as no ear position change between the defined sectors occurred. The proportion of forward, backward, and transverse ear postures (a)–(c), was then calculated in relation to the number of all observed ear posture changes. Left ear forward (d) was defined as the proportion of ear postures where the left ear was in a more forward sector than the right ear divided by the number of postures in which any of the ears was in a more forward sector than the other ear. The total number of ear posture changes (e), that is, the number of times one of the ears crossed one of the critical angles, was taken as the measure for the activity of the ears, that is, for ear movements.

While the previous definitions reflected the relative frequency of the ear postures, the new definitions reflected the relative duration for each posture such that any posture

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shown for longer durations at any one time was weighted accordingly. Thus, the relative duration seemed to be a better measure than the relative frequency.

Statistical analysis

To compare the two sets of definitions of ear postures and movements, the values obtained based on the new definitions were correlated with the values obtained based on the previous definitions. Due to the distribution of the recorded data and due to the nested structure of the data set, this correlation was conducted in several steps. To achieve a more normal distribution of our data, we transformed the original data (Figure 3, column I). All proportion variables (backward, transverse, forward, and left ear forward) were logit-transformed, and the movements of the ears were log-transformed (Figure 3, column II). To suppress potential variance between individuals and to account for the fact that data from the same individual were dependent, residuals were calculated based on a mixed-effects model including the intercept as the fixed effect and the individual sheep as the random effect (Reefmann *et al* 2009b; Figure 3, column III). Using these data, a correlation could still be caused largely by the expected differences between stimuli rather than by a more fine-grained correlation independent of the stimuli. Therefore, a second set of residuals was calculated based on a mixed model with the intercept as the fixed effect and test day nested within sheep as the random effect (Figure 3, column IV). A graphical analysis showed that both sets of residuals followed a distribution close to normal for all variables and, therefore, we used a Pearson product-moment correlation to compare the values based on our new definitions with the values based on the previous definitions. The sample size used was 864, that is, the values of 24 sheep confronted twelve times each with three different stimuli. Given this large sample size, all correlation coefficients were highly significant ($P < 2.2–16$), and therefore the focus of our results is on the actual size of correlation coefficients and their confidence intervals. We used the methods lme and cor.test in R (R Core Team 2013).

Results

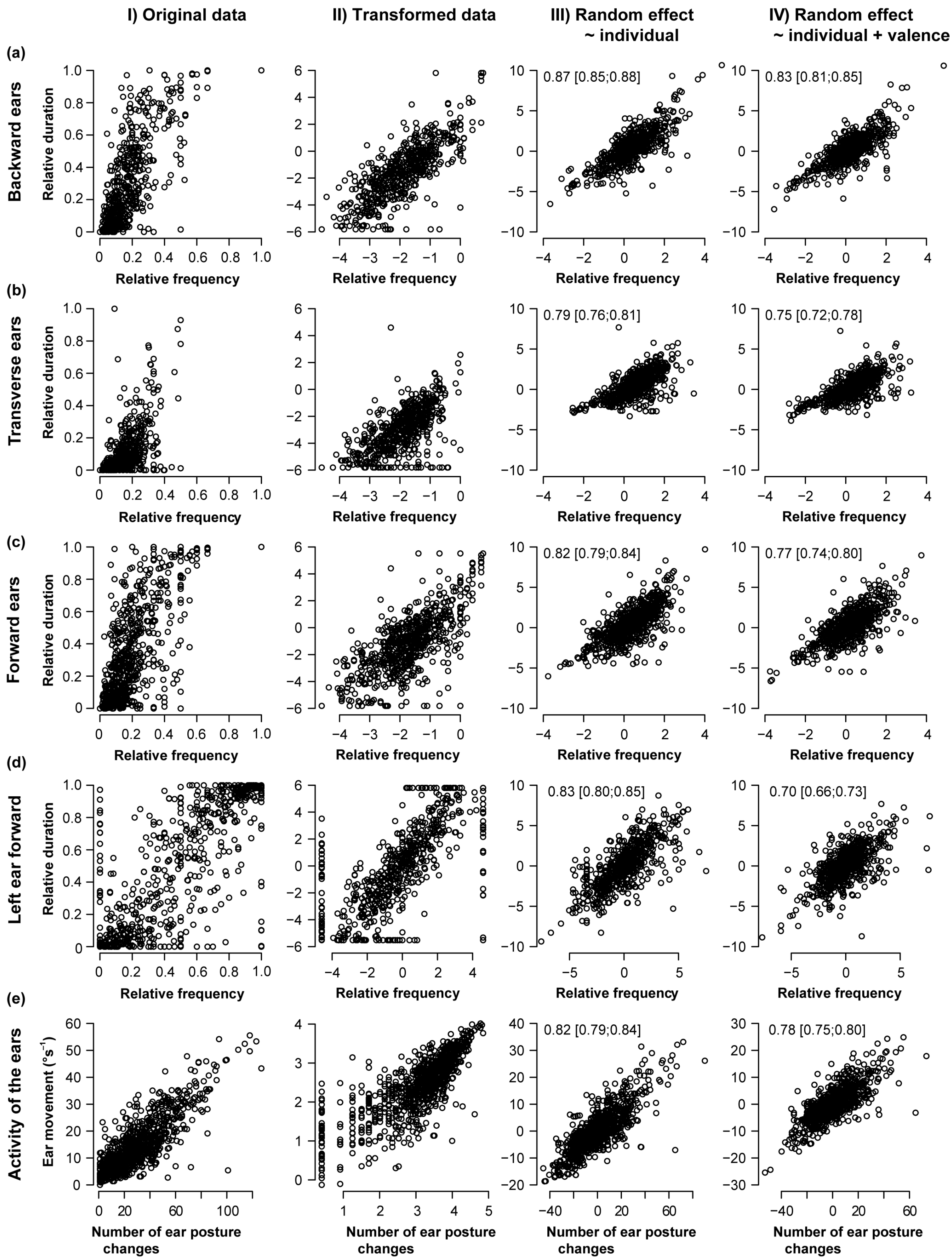
All correlations between the ear posture and movement values based on the new and previous definitions were greater than 0.70 (Figure 3, columns III and IV). After transformation of the data and calculation of residuals accounting for the dependencies in the data, the relationship was very close to linear (Figure 3, columns II–IV). The correlation coefficients (95% confidence interval) for the residuals accounting for the effect of the individuals were higher for all definitions (backward ears: 0.87 [0.85,0.88]; transverse ears: 0.79 [0.76,0.81]; forward ears: 0.82 [0.79,0.84]; left ear forward: 0.83 [0.80,0.85]; ear movements: 0.82 [0.79,0.84]; Figure 3, column III) than the coefficients additionally accounting for the different types of stimuli, if only slightly so (backward ears: 0.83 [0.81,0.85]; transverse ears: 0.75 [0.72,0.78]; forward ears: 0.77 [0.74,0.80]; left ear forward: 0.70 [0.66,0.73]; ear movements: 0.78 [0.75,0.80]; Figure 3, column IV).

Discussion

We compared values for ear postures and movements based on new definitions of relative durations facilitated by the continuous, automated tracking of ear positions with simpler values of relative frequency, which also were tracked by the automated system and calculated according to previous definitions used for video-based analyses in sheep (Reefmann *et al* 2009a,b). Overall, we found a high to very high correspondence in the values from the two definitions as measured by correlation coefficients. We found slightly higher coefficients when we accounted for the different individuals only and not for the different experimental stimuli as well. This can be explained easily because the different stimuli were thought to cause different emotional reactions in the sheep. These reactions are often visible in ear postures and movements (Reefmann *et al* 2009a,b; Boissy *et al* 2011). In consequence, the higher correlation coefficient could result from the variability of the ear posture measures that was somewhat larger across the three different stimuli than within stimuli. Nevertheless, even when the different stimuli were accounted for, correlation coefficients were still high (0.70–0.83) indicating good correspondence in the values for ear postures and movements within the different experimental situations.

Our results demonstrate that the new definitions for ear postures and movements obtained via the automated and continuous tracking of ear positions can be recommended for use. Moreover, they can replace the definitions of relative frequencies of the ear postures used in previous video-based analyses (as for example in Reefmann *et al* 2009a,b or Reimert 2013), because values calculated based on the two sets of definitions were very similar. This means that recent results based on relative durations can be compared with former results based on relative frequencies and that previous measures based on frequencies of ear postures should also be comparable to previous uses of (relative) durations as, for example, in Boissy *et al* (2011). In our view, the new definitions based on the continuous tracking are to be preferred because, conceptually, it makes sense to ask how much time (what proportion of time) animals spend in certain states if these states are thought to reflect their emotional reactions. This definition ensures that postures are weighted more heavily when they are shown for a longer time, which does not correspond to the (relative) frequency with which the postures are shown if the postures systematically vary in how long they are shown. Therefore, proportions of time seem to be a more adequate measure than the scaling of specific ear postures against the total number of ear postures observed. In addition, the automated tracking system is accurate in respect to both time-resolution and estimation of the angles between the two ears and the longitudinal axis of the head, aspects that cannot be approached using visual systems and human coding. Moreover, another significant advantage of the tracking system is the automatisation of the measurements and the data analysis. While video analyses are very time consuming (Martin & Bateson

Figure 3



Scatterplot matrix of measurements obtained from the automated, continuous tracking system comparing values of ear postures and movements based on their relative duration (on the Y-axis) with the values based on their relative frequency (on the X-axis). Each row depicts one ear posture: (a) backward ears; (b) transverse ears; (c) forward ears; (d) left ear forward and ear movements, ie (e) activity of the ears. The four columns indicate the four steps of analysis: (I) raw data; (II) transformed data (logit in [a]–[d]; log in [e]); (III) residuals corrected for between-subject variability with the individual as random effect, calculated from (II); (IV) residuals corrected for between-subject and between-stimulus variability with the individual and stimulus valence as random effects, calculated from (II) (see text for further explanation). Numbers indicate correlation coefficients including the 95% confidence intervals in square brackets.

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1993), the tracking system records all ear postures and movements automatically during the test once the system is installed and configured. The system can be set up within a time-period of about a day after some instruction. After an initial effort to code an evaluation software, the subsequent data analyses can then be performed easily and quickly after the experiments and simultaneously for all the tested animals. In contrast to video analyses, evaluation time is shorter, and no issues with inter-observer reliability arise. Finally, the system can be used for other purposes at the same time, for example, in our experiments, we simultaneously tracked general movement activity of the sheep in the test pen (Vögeli *et al* 2014).

However, some restrictions may apply to using the automated tracking system. Because the system is based on the reflection of markers detected by four infra-red cameras, the part of the animal which one wants to record has to be continuously visible to the cameras independent of the animal's postures or movements. The base system is dimensioned for an observation area of approximately 3 × 3 × 2 m (length × width × height). This area can be extended by using additional cameras (up to 16 cameras). In respect to measuring ear postures and movements, this is nevertheless not a real restriction in comparison to using video because, with the latter, the area that can be covered for such detailed analyses is also quite small. Barriers in the test area or multiple animals tested at the same time can lead to missing data caused by interferences between cameras and targets. The second issue is linked to the detection of the reflective markers by at least two cameras and the problem in assigning ear targets, that is, single marker balls, when more than one animal is tested at the same time. Sunlight also contains infra-red light and can therefore interfere with the correct recognition of the markers. Hence, data recordings should be conducted without direct sunlight, preferentially indoors. In short, this automated tracking system is specifically valuable in experimental settings and, in this respect, it is similar to a video-based system. A last point of concern may be the fact that the tracking markers have to be attached to the animal. However, with large animals that are used to wearing head-collars and/or are tagged with ear marks, as are our sheep, this is no real issue after some habituation, especially because the tracking markers are very lightweight. In fact, in our study, no reaction to the tracking equipment was observed after a few days of habituation.

Animal welfare implications and conclusion

The automated tracking system evaluated here provides both a higher accuracy and a simpler method of measuring ear postures and movements in comparison to video analyses, and it can be recommended highly for indoor experimental procedures. The new method can be a very useful instrument for studies relying on the evaluation of ear postures and movements for the understanding of emotional reactions in sheep and other similarly sized animals.

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