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Animals remember previous facial expressions that specific humans have exhibited

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Summary

For humans, facial expressions are important social signals and how we perceive specific individuals may be influenced by subtle emotional cues that they have given us in past encounters. A wide range of animal species are also capable of discriminating the emotions of others through facial expressions [1–5], and it is clear that remembering emotional experiences with specific individuals could have clear benefits for social bonding and aggression avoidance when these individuals are encountered again. While there is evidence that non-human animals are capable of remembering the identity of individuals who have directly harmed them [6,7], it is not known whether animals may form lasting memories of specific individuals simply by observing subtle emotional expressions that they exhibit on their faces. Here we conducted controlled experiments in which domestic horses were presented with a photograph of an angry or happy human face and several hours later saw the person who had given the expression in a neutral state. Short-term exposure to the facial expression was enough to generate clear differences in subsequent responses to that individual (but not to a different mismatched person), consistent with the past angry expression having been perceived negatively and the happy expression positively. Both humans were blind to the photograph the horses had seen. Our results provide clear evidence that some non-human animals can effectively eavesdrop on the emotional state cues that humans reveal on a moment-to-moment basis, using their memory of these to guide future interactions with particular individuals.

Results & Discussion

As well as recognising individual conspecifics and humans [8,9] and discriminating between different facial expressions in both species [2,10], horses can also learn to differentiate unknown people based on facial features alone and transfer this discrimination from photographs to live models [11]. In the current study we first presented horses with a photograph of a happy or angry face belonging to one of two human models for 2 minutes. Half the subjects saw the happy or angry face of model 1 and the other half the happy or angry face of model 2 (Figure 1; see Table S1 for details of response in this exposure phase). Several hours later, the horses in the experimental group were presented with the live model previously depicted in the photograph, but this time adopting a neutral expression. Critically, the live models were blind to the emotional valence of the photograph that the horses had previously seen. In order to determine whether any memory of the past emotional encounter was specific only to the individual seen adopting this expression, a control group was presented with a different, mismatched, live person (the other model in the experimental group), who was also adopting a neutral expression. Thus, in our design, not only was each individual model presented both as a happy and angry variant in the photographs, but the models also acted as live mismatch controls for each other – matching the identity of the person in the photographs in the experimental trials and contrasting with it in the control trials. If horses remember a single brief exposure to an emotional facial expression of a particular individual, then we would expect the experimental group to react either positively or negatively to the neutral model based on which facial expression they had previously seen, but the mismatch control group not to differ in response.

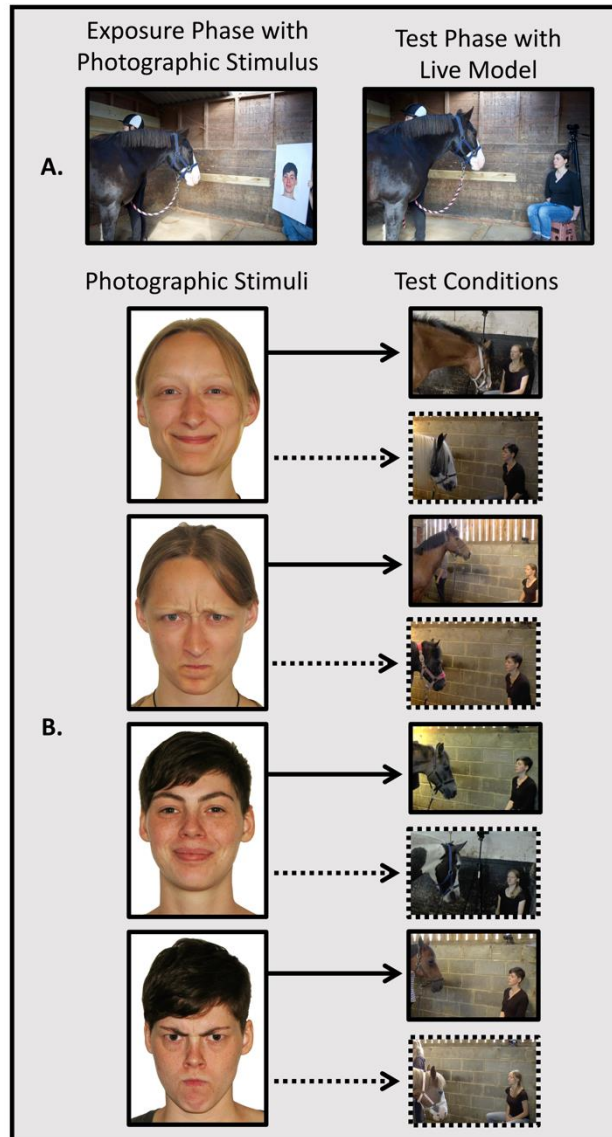


Figure 1. Diagram of the experimental design and set up. A. Images of the experimental set up in exposure phase (left panel) and test phase (right panel). B. Photographic stimuli presented in the exposure phase in relation to the permutations of the test phase. In the exposure phase, each horse was presented with a photograph of Model A or Model B either happy or angry. In the test phase, subjects in the experimental group were presented with the live model previously depicted in the photograph, but this time adopting a neutral expression. Subjects in the mismatch control group viewed the previously unseen model adopting a neutral expression. The solid border around the images of the test phase denotes the experimental group and the dashed border denotes the mismatch control group. Both models were blind to the

original condition (happy or angry). The presentation duration, stimulus movement and post-test period was the same in both presentation and exposure phases. See also Table S1 for details of exposure phase.

Looking behaviours (lateralised and binocular looking), displacement and stress behaviours, approach, avoidance and heart rate measures were recorded to evaluate responses to the neutral person and gain information on the subjects' emotional state. Lateralized responses provide a useful window into what animals are experiencing [12]. Across a wide range of species including horses, negative and potentially threatening stimuli tend to be preferentially processed in the right hemisphere, indicated by a left gaze bias, and more pro-social stimuli in the left hemisphere, indicated by a right gaze bias [12–14] and lateralised responses to human facial expressions have been observed in dogs and horses [2,15]. In our study, there was a significant difference in the first gaze biases of horses between the positive and negative groups ($N = 21$, $p = 0.008$), with the subjects that had previously seen the negative photograph showing an initial left gaze bias and those that had seen the positive photograph no gaze bias (negative: $N = 11$, $K = 10$, $p = 0.01$; positive: $N = 10$, $K = 3$, $p = 0.34$). In addition, subjects originally shown the negative photo spent significantly more time overall looking at the live model with their left eye (mean = $15.2s \pm 4.3$ S.E.) than the subjects shown the positive photo (mean = $3.6s \pm 0.9$ S.E., $t_{1,22} = 2.67$, $p = 0.02$, $r = 0.49$; see Figure 2A). The opposite is true when we look at right gaze bias time – subjects previously shown the happy photo spent more time viewing the model with their right eye than the subjects shown the angry photo (mean = $7.5s \pm 2.9$ S.E. vs. $0.7s \pm 0.3$ S.E., $t_{1,22} = 2.33$, $p = 0.04$, $r = 0.44$; see Figure 2B). These results are also reflected in significant differences between standard laterality indices following the angry versus happy presentations (mean = 0.36 ± 0.07 S.E. vs. -0.07 ± 0.06 S.E., $t_{1,22} = 4.06$, $p = 0.001$, $r = 0.65$; see Figure 2C below). The response to the neutral person showed a significant right hemisphere bias following the presentation of the angry photograph ($t_{11} = 4.12$, $p = 0.002$, $r = 0.78$) and no significant bias following the presentation of the happy photograph ($t_{11} = -1.16$, $p = 0.27$). In contrast to these laterality biases in the experimental group, there were no significant differences in lateralised looking

behaviours in the mismatch control group as a function of whether subjects had previously seen an angry or happy photograph (see Table S2). The time that horses spent looking directly at the models previously portrayed as happy versus angry was not significantly different in either the experimental (mean = $30.5s \pm 5.7$ S.E. vs. $26.2s \pm 5.9$ S.E., $t_{1,22} = 0.52$, $p = 0.61$) or the mismatch control groups (see Table S2).

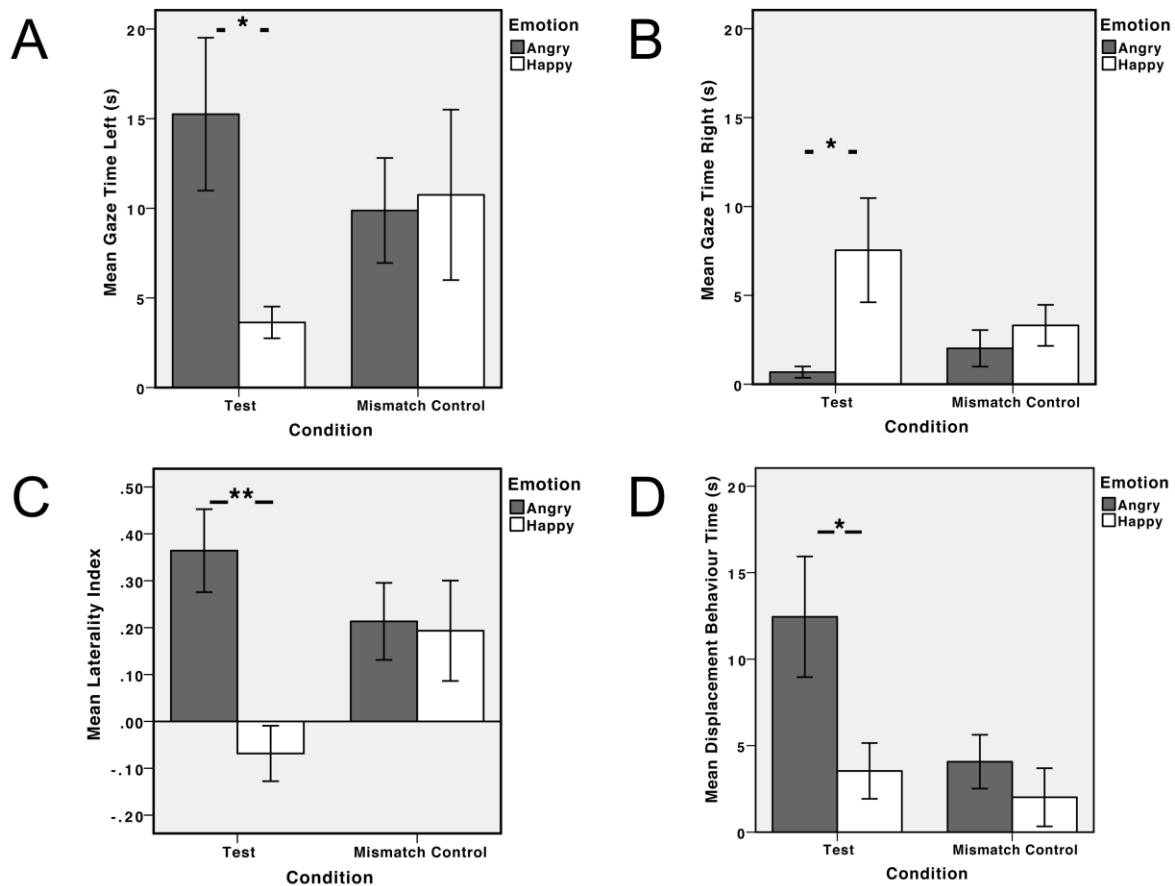


Figure 2. Responses of the experimental and control groups to the neutral person after viewing the happy versus angry photographs (Mean $\pm$ S.E.M.). Graphs show A) Time spent viewing the stimuli with a left gaze bias B) Time spent viewing the stimuli with a right gaze bias C) Laterality indices $LI = (L-R)/(L+M+R)$, where L, M, and R represent time spent looking left, right, and in the middle. Positive scores indicate a left-gaze bias

and negative scores a right-gaze bias D) Time spent engaging in displacement behaviours. $N = 48$, * $p < 0.05$; ** $p < 0.001$. See also Tables S2 and S3.

Displacement behaviours are actions that appear unrelated to the current situation, such as scratching, and are thought to be a coping mechanism in stressful situations. These behaviours may also provide observable insights into how animals are perceiving emotional expressions [16]. Horses in the experimental group that had been shown the angry photo spent more time engaging in displacement behaviours (scratching, floor sniffing and a performing a species-specific behaviour termed lick and chew) when viewing the live neutral person than those that had been shown the happy photo (mean = $12.5s \pm 3.5$ S.E. vs. $3.6s \pm 1.6$ S.E., $t_{1,15.5} = 2.30$, $p = 0.036$, $r = 0.42$; see Figure 2D). Looking at additional stress-related behaviours, only one horse showed avoidance behaviours and two showed nostril dilation during the test (all in the angry condition), thus no statistical analyses were performed on these measures. There were also no differences in the number of horses that approached the live model (Fisher's Exact Test (FET) $N = 23$, $p = 0.68$). In the mismatch control group, there were no significant differences in any of the above behavioural variables as a function of whether subjects had previously seen an angry or happy person in the photograph (see Table S2). Furthermore, no differences in heart rate measures were found between the two conditions in the experimental group, although all values were in the predicted direction of higher Heart Rate (HR) and lower Heart Rate Variability (HRV) for the angry condition (See Table S3 for details).

Using the facial expressions of others to gauge the correct response to those individuals in the future requires a combination of cognitive abilities including sensitivity to emotional facial expression, identification of the individual, and memory for specific emotional events. Our results demonstrate that some animals are capable of taking into account a single encounter with an individual displaying an emotional facial expression when subsequently interacting with that same individual in a neutral context three to six hours later. This result is particularly striking because the horses did not have a strongly positive or negative direct experience with the person – they merely viewed a

photograph of them with either a happy or angry facial expression. This short-term exposure to a facial expression was enough to generate clear differences in subsequent lateralized looking and levels of displacement behavior that were consistent with the angry expression being perceived negatively and the happy expression positively.

It is notable that the mismatch control groups – where the subjects saw a different live person to the one seen in the photographs – showed an overall left gaze/right hemisphere bias. This bias may be driven by a number of factors including the activation of right hemisphere face processing centres or the subjects perceiving the experimental set up negatively [17,18]. Our difference between the two test groups (angry versus happy) could therefore be driven by the response to the happy generating a reduction in left gaze as well as by the angry producing an increase in left gaze/reduction in right gaze. The extent to which differences in response are driven primarily by a positive response to happy or a negative response to angry (or a combination of the two) would be an interesting area for future research. In addition, there are a number of possible (non-mutually exclusive) factors that may give rise to horses' abilities to remember transient human facial expressions and use these expressions to guide subsequent interactions. Horses may have an innate ability to remember the facial expressions of conspecifics, and this ability automatically extends to humans. Alternatively, the ability could have specifically evolved during the process of domestication or may be learnt during a lifetime of experience with people. Further work could usefully assess the evolutionary and ontogenetic mechanisms involved, by comparing the responses of domestic and wild species, as well as individuals with varying degrees of human exposure.

A powerful aspect of our research is that the horses were not re-exposed to the negative or positive stimuli, rather they were presented with the neutral person who was blind to the valence of the photograph that subjects had previously seen. Thus the results could not be due to emotional contagion, i.e. the subjects could not be picking up on the emotion expressed by the live human model. Instead, our results suggest the subjects were using a memory of the positive or negative expression in the specific human previously seen to guide their response to that same person even when they

adopted a neutral expression. It has been suggested that facial expressions in primates, and perhaps across mammals generally, represent adaptations to living in complex societies where it is important to remember multiple individuals and quickly perceive intent and information about their emotional state [19]. Indeed appropriate discrimination of facial expressions and the underlying emotions is seen to be central to social competence in humans [20]. Testing the generality of such a social competence hypothesis requires a wide-ranging phylogenetic comparison of the production and comprehension of emotional and identity based facial signals in primate and non-primate species with varying degrees of sociality. Our paper provides direct evidence of a key role for processing of facial cues to emotion in long-term social functioning in a non-primate, throwing light on its adaptive significance across species and indicating that facial expressions can be registered and remembered even in inter-specific communication.

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Author Contributions

Conceptualization, K.M., L.P., K.G. and A.V.S.; Methodology, K.M., L.P., K.G. and A.V.S.; Investigation, L.P., A.V.S. and K.G.; Formal Analysis, K.M., L.P. and K.G.; Writing, K.M., L.P. and K.G., Funding Acquisition, K.M. and L.P.; Supervision, K.M. and L.P.

Declaration of Interests

The authors declare no competing interests.

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STAR Methods

Contact for Reagent and Resource Sharing

Further information and requests for resources should be directed to and will be fulfilled by the Lead contact, Karen McComb (karenm@sussex.ac.uk).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Supplementary Information		
Raw Data	Sussex Research Data Repository (SURE)	www.sussex.figshare.com .
Experimental Models: Organisms/Strains		
<i>Equus caballus</i>	4 Equestrian Centres in East Sussex, UK.	
Software and Algorithms		
SPSS Version 23	IBM	https://www.ibm.com/analytics/data-science/predictive-analytics/spss-statistical-software

Experimental Model and Subject Details

48 horses of various breeds from 4 locations in East Sussex, UK, participated in the study (13 females, 35 gelded males, 3 – 29 years, mean = 15.15 ± 6.08 S.D.). 24 participated in the experimental condition and 24 in the control condition. Subjects were either riding school horses or owned by private individuals and all were accustomed to regular human handling. Testing occurred in a stable in their familiar environment alongside their normal daily routines. The experimental condition trials were conducted during June – July 2015 and the control condition trials during June – July 2016.

Ethical Statement

This research adheres to the Association for the Study of Animal Behaviour (ASAB) guidelines for the use of animals in research and was approved by the University of Sussex Ethical Review Committee (Reference Number: Non-ASPA 3-Jan2014).

Owners of the horses gave prior consent for participation and were notified that they could withdraw their consent at any time. Horses remained within their familiar environment and were not food deprived.

Method Details

Stimuli

2 female experimenters were pictured for the photographic stimuli in the exposure phase and subsequently used as the live models in the test phase for both the experimental and control trials. Photographs of each model expressing a happy and angry facial expression were taken (see Figure 1). A certified Facial Action Coding System (FACS) coder documented the facial movements (AUs) present in each stimulus [21]. For the happy expressions, these included AU12 (lip corner puller) and AU6 (cheek raiser) and for the angry expression these included AU4 (brow lowerer), AU17 (chin raiser) and AU24 (lip pressor). The images were printed in colour on A3 (420 x 297mm) paper, laminated and then fixed on to an A1 (594 x 841mm) white board for presentation (see Figure 1). The slightly supra-normal size of the images and the larger white board were used to increase their salience. Photographs were used in the exposure phase to ensure standardised presentation of emotional expression across context. During the test phase, each live model wore a black shirt and blue jeans. They were instructed to relax their facial muscles and to adopt a neutral expression, eyes looking forwards but not focused on the horse, in order to keep their expression “soft” and to prevent a stern expression developing.

Procedure

All trials were conducted in a test stable cleared of bedding and hay. During the exposure phase a small cushion was placed in the presentation corner to allow the photograph presenter (who was concealed behind the photograph board during the presentation) to comfortably kneel when necessary to obtain the correct presentation height. Similarly, in the test phase a small stool was placed in the presentation corner to allow the live model to sit at the correct height during presentation. The study thus

consisted of 2 phases, an initial presentation of the photographic stimuli (depicting either a happy or angry model) followed by a second phase in which the live model depicted in the photograph was presented. The methodology employed in the exposure and test phases was designed to be as similar as possible. 3-6 hours elapsed between the exposure and test phase, during which time the horses were returned to their stable or field. No horses were ridden during the interval to ensure accurate heart rate readings in the test phase. The handler, photograph presenter and the live models were blind to the emotion presented in the exposure phase. The responses of the horses were recorded on two Panasonic cameras (X920 and HC-V750 with a wide angled lens) positioned to the side and front of the experimental area (for experimental set up see Figure 1).

Exposure phase. Each horse was led in to the experimental stable and allowed to move freely on a loose lead rope before the start of the presentation. After 2 minutes the stimulus presenter entered and moved to the location where the stimulus was to be presented. The presentation board was initially held facing the wall with the top in line with the horse's withers (top of their shoulders) to ensure a standard presentation height. The handler positioned the horse so that they were facing forwards, 1m from where the stimulus was to be presented. The handler stood at the horse's left shoulder facing the rear of the horse so they could not see the stimulus. The horse was held on a loose lead rope at a length of 1.5m allowing free movement within that range. After a minimum of 30s, once the horse was settled in position, the handler verbally indicated that the presentation could begin. The presenter then turned the presentation board to face the horse, keeping their own face hidden behind the board and held it in position for 20s before moving the board forward 10cm and holding this position for a further 20s. The board was then returned to the original position and this cycle was repeated 2 more times so that the stimulus was presented for 2 minutes. The presenter marked the end of the presentation verbally, turned the photograph towards the wall and exited the stable with the photograph still hidden from the subject and handler's view. The slight forward and backward movement of the stimulus was included to increase the subject's

attention to the presentation. The subject was then held on a loose lead rope for a further 2 minutes before exiting the stable and being returned to their own field/stable.

Test phase. Subjects' heart rate was recorded using a Polar Equine RS800CX heart rate monitor. Once the monitor was attached, this phase followed the same protocol as the exposure phase with the exception that instead of presenting a photograph, the live model entered the stable facing away from the horse and when the subject was in position, turned to sit on a stool facing the horse, adopting a neutral expression. The presentation duration, stimulus movement and post-test period was the same as the exposure phase.

For subjects in the experimental condition, the live model was the same person as depicted in the photograph presented in the exposure phase. For the mismatch control subjects, if person 1 was depicted in the photograph, person 2 would be the live model and vice versa. The presentation of emotion, model and condition (mismatch control/experimental) was counterbalanced across subjects.

Quantification and Statistical Analysis

Heart Rate Processing

Data were uploaded from the receiver watch to Polar ProTrainer 5 Equine Edition software (Polar UK, Warwick, UK) and then imported as .txt files into Kubios HRV (v. 2.2; Biosignal Analysis and Medical Imaging Group, University of Eastern Finland, Kuopio, Finland). Custom artefact correction at 0.3, smoothed with a Lambda value of 500 was applied, and RMSSD heart rate measures were obtained for statistical analysis. The listings of each heart beat value were also extracted from Polar

ProTrainer and the modal and maximum values were calculated in excel. One subject was excluded because there were >5% corrections in the Heart Rate file.

Behavioural Coding

The responses of the subjects to the stimuli were analysed for the exposure and test phases. The amount of time spent looking binocularly and with a left and a right gaze bias as well as avoidance and approach behaviours were coded. Displacement behaviours including scratching, floor sniffing and lick and chew (a species-specific behaviour) were included as indices of stress. Nostril dilation was also coded as an index of negative emotion. Detailed descriptions of each behaviour are given in Table 1. HR measures during pre-test, test and post-test were calculated for the experimental condition in the test phase to gain insights into the physiological response of subjects to the live model. 1 subject had to be removed from HR analyses due to a high rate of errors in the file (10.7%). Although the full 2 minute presentation was initially captured, when reviewing the videos it was clear that many of the subjects became disinterested in the model before the end of the test. Consequently, data from only the first minute were used to provide a more accurate representation of the subjects' responses to the stimuli.

Table 1. Details of Behavioural Coding and Heart Rate Measures.

Behaviour	Coding scheme definition
Looking durations	
<i>Direct Gaze</i>	Horse's head is directed centrally towards the stimulus.
<i>Gaze Bias (Left/Right)</i>	The horse is attentive to the stimulus with its head turned more than 15° to the left or right. Attentiveness is determined by the horse having at least one ear and/or eye focused on the stimulus.
Approach and avoid	
<i>Approach</i>	Horses were coded as having approached the stimulus (Y/N) if their nose came within 30cm of the stimulus.
<i>Avoid</i>	Any increase of distance from the stimulus combined with indices of stress (e.g. nostril dilation, startle, high muscle tone) was coded as avoidance behaviour. The total duration of these behaviours at any distance to the stimuli was recorded.
Displacement and stress behaviours	
<i>Scratching</i>	Subject scratches themselves with their nose/teeth or scratches against something. A minimum 2s pause is required to code a new bout.
<i>Floor sniffing</i>	The horse remains attentive to the stimulus while the head is lowered to the ground, with their nose within 10cm of the floor. Attentiveness is determined by the horse having at least one ear and/or eye focused on the stimulus.
<i>Lick and chew</i>	Horse chews and protrudes tongue with no external stimulus as a cause, e.g. not chewing hay or biting wood [22]. A minimum 2s pause is required to code a new bout.
<i>Nostril dilation</i>	The skin above the nostrils is inflated as the air is blown outwards; generally driven by strong exhalation/blowing [23].
Heart Rate Measures	
<i>HR Mode</i>	Modal heart rate value.
<i>HR Max</i>	Maximum peak of heart rate.
<i>RMSSD</i>	Square root mean of successive beat to beat (RR) differences.

Statistical Analysis

The number of horses looking to each side in their first gaze bias was assessed using binomial probability (two-tailed) for each test condition and compared across conditions using 2X2 Fisher's Exact Tests (subjects not showing a gaze bias were removed from analysis). Independent t tests with emotion as the IV were performed on the data to assess differences in duration of displacement behaviours and looking time between the positive versus negative conditions (left, right and binocular looking durations). In addition, a laterality index (LI) for total looking time was calculated: $LI = (L - R) / (L + M + R)$, where L, M, and R represent time spent looking left, right, and in the middle. Positive scores indicate a left-gaze bias and negative scores a right-gaze bias. The indices for the positive and negative conditions were compared using an independent t test (where there was not homogeneity of variance, adjusted significance values were used). Deviations from binocular gaze (chance level: 0) for each condition were measured using one-sample t-tests to determine the extent of the lateralised response to each emotion independently. The number of horses that approached and avoided the stimuli as well as the number that showed nostril dilation was compared across conditions using 2X2 Fisher's Exact or Chi Square Tests. Where sufficient subjects performed these behaviours, comparison of behaviour durations were also assessed using independent t tests. One horse was removed from the approach analyses due to experimenter error and there were missing values for the left gaze duration of one subject. The modal and maximum heart rate of subjects and well as their heart rate variability as measured by RMSSD, during the test, post-test, and the change from baseline to test were compared across conditions using a series of independent t tests. Behavioural responses were blind coded by KG frame by frame using Sportscore Gamebreaker Plus® 7.5.5 software (www.sportstec.com). 21% were second coded providing a high degree of inter-observer reliability (see Table 2).

Table 2. Table of inter-observer reliability for behavioural coding. Measures of duration were analysed using Intra-class Correlation Coefficients (ICC) and categorical measures were analysed using Cohen's Kappa.

Behaviour	ICC	Lower bound	Upper bound	F	P
Direct Looking	0.95	0.88	0.98	38.53	<0.001
Gaze Bias Right	0.97	0.91	0.99	62.47	<0.001
Gaze Bias Left	0.92	0.80	0.97	22.28	<0.001
Displacement	0.996	0.990	0.998	569.48	<0.001
Avoidance	0.93	0.84	0.97	28.80	<0.001
Approach	0.88	0.73	0.95	15.51	<0.001
Nostril Dilate	0.94	0.86	0.98	34.67	<0.001
Behaviour	κ			t	P
First Look	0.81			4.41	<0.001
Approach Y/N	0.87			3.99	<0.001
Avoid Y/N	0.86			3.87	<0.001
Dilate Y/N	0.74			3.42	0.001

Data Availability

The raw data from the experimental and exposure phases are available at the Sussex Research Data Repository (SURE) at www.sussex.figshare.com.