Horses feel emotions when they watch positive and negative horse–human interactions in a video and transpose what they saw to real life

Miléna Trösch, Sophie Pellon, Florent Cuzol, Céline Parias, Raymond Nowak,
Ludovic Calandreau, Léa Lansade

► To cite this version:

Miléna Trösch, Sophie Pellon, Florent Cuzol, Céline Parias, Raymond Nowak, et al.. Horses feel emotions when they watch positive and negative horse–human interactions in a video and transpose what they saw to real life. *Animal Cognition*, 2020, 23 (4), pp.643 - 653. 10.1007/s10071-020-01369-0 . hal-03038684

HAL Id: hal-03038684

<https://hal.science/hal-03038684>

Submitted on 3 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution| 4.0 International License

Horses feel emotions when they watch positive and negative horse–human interactions in a video and transpose what they saw to real life.

Miléna Trösch^{a*} (milena.trosch@gmail.com), Sophie Pellon^a (pellon.sophie@gmail.com), Florent Cuzol^a (florent.cuzol@gmail.com), Céline Parias^a (celine.parias@inrae.fr), Raymond Nowak^a (raymond.nowak@inrae.fr), Ludovic Calandreau^a (ludovic.calandreau@inrae.fr), Léa Lansade^a (lea.lansade@inrae.fr).

* corresponding author

^a INRAE, PRC, CNRS, IFCE, Université de Tours, 37380 Nouzilly, France.

Keywords: *Equus caballus*, Social cognition, Social eavesdropping, Human-animal relationship, Emotional contagion

Abstract

Animals can indirectly gather meaningful information about other individuals by eavesdropping on their third-party interactions. In particular, eavesdropping can be used to indirectly attribute a negative or positive valence to an individual and to adjust one's future behavior towards that individual. Few studies have focused on this ability in nonhuman animals, especially in nonprimate species. Here, we investigated this ability for the first time in domestic horses (*Equus caballus*) by projecting videos of positive and negative interactions between an unknown human experimenter (a “positive” experimenter or a “negative” experimenter) and an actor horse. The horses reacted emotionally while watching the videos, expressing behavioral (facial expressions and contact-seeking behavior) and physiological (heart rate) cues of positive emotions while watching the positive video and of negative emotions while watching the negative video. This result shows that the horses perceived the content of the videos and suggests an emotional contagion between the actor horse and the subjects. After the videos were projected, the horses took a choice test, facing the positive and negative experimenters in real life. The horses successfully used the interactions seen in the videos to discriminate between the experimenters. They touched the negative experimenter significantly more, which seems

counterintuitive but can be interpreted as an appeasement attempt, based on the existing literature. This result suggests that horses can indirectly attribute a valence to a human experimenter by eavesdropping on a previous third-party interaction with a conspecific.

Introduction

Social animals can indirectly gather meaningful information from other individuals by eavesdropping on their third-party interactions (i.e., “social eavesdropping”; Bonnie and Earley 2007). This social eavesdropping has been observed in various species and contexts. For instance, in the context mate choice, birds and fishes can eavesdrop on interactions to assess the quality of a potential partner (Mennill et al. 2002; Ophir and Galef 2004; Valone 2007; Aquiloni and Gherardi 2010; Kavaliers et al. 2017). Several species of birds, fishes and rodents observe conflictual interactions to judge the relative quality and dominance rank of the opponents (Peake et al. 2001; Earley and Dugatkin 2002; Höjesjö et al. 2007; Valone 2007; Lai et al. 2014). Furthermore, social eavesdropping can also allow animals to attribute a negative or positive valence indirectly to unknown individuals by two different mechanisms. First, individuals can observe the emotional reaction of a third-party person during an interaction with a stranger to adjust their own behavior towards that stranger; this has, for instance, been studied in dogs and human infants (Feinman and Lewis 1983; Murray et al. 2008; Freidin et al. 2013; Duranton et al. 2016). Second, animals can also indirectly attribute a valence to unknown individuals based on how the individuals behaved towards a third party and to use this information to adjust their own behavior towards the individuals accordingly. This latter ability has been investigated in a few non-human species, including great apes, capuchin and marmoset monkeys, domestic dogs and reef fish (see Abdai and Miklósi 2016 for a review). Chimpanzees, dogs and marmoset monkeys were, for instance, shown to prefer eating food offered by an experimenter who previously gave food to a human beggar more than food offered by an experimenter who refused to do so (Russell et al. 2008; Subiaul et al. 2008; Marshall-Pescini et al. 2011; Kawai et al. 2014). Similarly, capuchin monkeys and dogs showed a preference for an experimenter who had previously helped open a container to retrieve an object (of neutral valence to the subject) compared to an unhelpful experimenter (Anderson et al. 2013; Chijiwa et al. 2015). A similar ability has also been studied in reef fish species in the context of interspecific mutualism with the cleaner fish (*Labroides dimidiatus*): reef fish were shown to eavesdrop on the interaction between these cleaner fishes and other third-party clients in order to select cooperative partners (that remove the ectoparasites from clients’ mouths and do not

63 cheat by eating the clients' mucus; Bshary and Grutter 2006). This underlies the importance of
64 carrying out studies in taxa other than dogs and primates in order to know how widespread this
65 ability to attribute a valence based on social eavesdropping is.

66 This valence attribution, often studied in the context of cooperation, has also been reported
67 during interactions involving emotional content. Nitzschner et al. (2012) showed dogs an
68 interaction between a conspecific and a "nice" experimenter—paying attention to and petting
69 another dog—and an "ignoring" experimenter who ignored the other dog. As cooperation is a
70 complex mechanism that implies high cognitive demands (Cheney 2011), this alternative
71 paradigm can be particularly useful to study a broader range of species, especially species that
72 are not known to cooperate.

73 In this study, we investigated for the first time whether domestic horses (*Equus caballus*) could
74 indirectly attribute a valence to a human by eavesdropping on an interspecific social interaction.
75 We used a paradigm based on emotional interactions, as the expression and processing of
76 emotions have been well described in this species. Horses can express emotions both vocally
77 (Briefer et al. 2017) and visually (by body posture and facial features; Lansade et al. 2018).
78 Typical facial expressions have been well described for both negative (Dalla Costa et al. 2014,
79 2017; Glerup et al. 2015) and positive (Lansade et al. 2018) emotions. Horses can recognize
80 facial expressions related to emotions in both humans (Smith et al. 2016; Nakamura et al. 2018;
81 Trösch et al. 2019a) and conspecifics (Wathan et al. 2016) and adjust their behavioral response
82 accordingly, even in a future encounter a few hours later (Proops et al. 2018). Moreover, horses
83 are both highly sociable (Ringhofer et al. 2017) and share a close relationship with humans in
84 their daily lives. They are very attentive to humans and their behavior (e.g., Proops et al. 2009;
85 Proops and McComb 2010, 2012; Lampe and Andre 2012; Proops et al. 2013; Malavasi and
86 Huber 2016; Ringhofer and Yamamoto 2016; Trösch et al. 2019b) and can learn socially from
87 a conspecific or a human demonstrator (Krueger et al. 2014; Rørvang et al. 2015; Schuetz et al.
88 2017). Hence, horses are good candidates for investigating whether they can also learn by
89 eavesdropping on interactions between a human and a third-party conspecific to attribute a
90 valence to the human.

91 To examine this, we showed the horses two kinds of interactions between an experimenter and
92 an unfamiliar "actor" horse: a positive interaction (a grooming session) and a negative
93 interaction (an unpleasant veterinary act). These scenes were presented to the horse through
94 silent videos projected in real size in front of each horse, and we investigated whether they
95 could perceive the emotional content of these scenes through behavioral and physiological
96 measurements. Then, the horses met the two experimenters in real life in a choice test. Our

hypotheses were the following: 1) Horses would express more positive emotions when watching the positive scene and more negative emotions in front of the negative scene. A control condition in which the tested horse observed the same videos but with the human and horse actors replaced by moving ovals allowed us to check that the horses were not simply reacting to a difference in movements between the two videos. 2) The horses would discriminate between the positive and negative experimenters during the choice test by seeking more contact with one of them.

Materials and methods

Subjects and husbandry

Forty-seven female adult Welsh ponies (mean age $\pm$ SE = 8.47 ± 0.61) were used for this study. Twenty-four of them were included in the test condition, and 23 were included in the control condition. These horses were bred and had lived all their lives at the experimental unit (PAO, doi: 10.15454/1.5573896321728955E12) of the French National Institute for Agricultural Research (INRA, Nouzilly, France). They were familiar with humans and being handled. During the experiment, the horses were housed indoors on straw bedding in groups. They were fed with straw and hay, with access to water *ad libitum*. None of the horses had been involved in a similar experiment before.

Ethical note

Our protocol was submitted to the Val de Loire Ethical Committee (CEEA VdL, France) and received a positive recommendation. Horses did not undergo any invasive procedure during the experiment. Horses lived in groups, went to an outside paddock on a daily basis, and were not food-deprived.

Videos

-Test condition

The experiment consisted of consecutively projecting two silent videos (one positive video and one negative one), each 30 s long, to a horse (Fig. S1). The positive video showed an experimenter grooming an “actor” horse. In this case, the actor horse expressed positive emotions (Table S1) through a typical facial expression that has been described before (Lansade et al. 2018): ears oriented backwards, low neck, lips extended and moving in a grooming attempt. The negative video showed a second experimenter (different from the first one)

performing a veterinary act on the horse: touching her ears to administer an ointment and applying a spray towards her head. We used a small air spray, which was completely hidden in the experimenter's hand, so that it was invisible on the video. In that case, the actor horse expressed fearful reactions by jolting and trying to run away from the experimenter (four times in each video). She adopted a typical facial expression of negative emotions (Table S1): elevated neck and eyes wide open with the sclera visible (Lansade et al. 2018).

The two experimenters (both women) and the actor horse were unfamiliar to the horses prior to this experiment. Each experimenter wore a specific color of clothes (light brown for one of them and dark blue for the other) and could be either positive or negative depending on the horse tested: the role of the two experimenters and the order of the videos were counterbalanced between the horses. The videos were recorded beforehand and in the same location. Importantly, the experimenters were asked to keep a neutral facial expression (not expressing any positive or negative emotions) in both videos, and the videos were later checked independently by three people and edited to delete the scenes in which this was not the case. The videos consisted in several short scenes following one another. The number and the duration of these scenes were identical in all videos: two scenes of 10 s, followed by three scenes of 2 s and then one last scene of 4 s. Moreover, all the videos were checked to ensure that the experimenters' face was entirely visible during at least 60% of the video.

-Control condition

In the control condition, the videos used during the test were edited so that the experimenters and the horse were replaced by two ovals with the same surface area and colored with the main color of the experimenter and the actor horse as appropriate. The videos were edited semiautomatically using the image editing and analysis software ImageJ and Fiji (Schindelin et al. 2012; Rueden et al. 2017). These ovals followed the movement pattern of the horse and the experimenter and copied the variations in their surface area (which was recalculated every 25 ms).

Set up and procedure

The entire experiment took place in a large, familiar stall (3.5 x 4.5 m; Fig. 1). The videos were projected onto a large white screen (2 x 2 m) by a DLP projector Acer P6200S. During the video projection, the horse was attached in front of the screen by two loose leading reins (one on each side of the horse). Moreover, an assistant stood next to the horse during the projection for security reasons in case the horse became distressed or risked hurting itself during the course

of the equipment (which never occurred during our study). The assistant had his or her back to the screen, was looking on the ground, and did not know which video was being projected in order to avoid unintentional social cueing.

The horses were tested individually in the following manner:

- Test condition:

The test condition was realized with 24 horses.

1. Familiarization: The horses were familiarized with the experimental set up, video projection and device used to measure their heart rate (cf. “Coding and statistical analyses”). Familiarization consisted of at least 3 sessions of 10 minutes each, with one session per day, typically on three consecutive days. More sessions were added if necessary until the horse could be secured and stayed still while in front of a video with a heart rate lower than 80 bpm during a period of more than 30 s. The videos used were specific to this familiarization and showed an unknown horse walking and running in a sand paddock. The test took place in the afternoon on the same day as the last familiarization session.

2. Projection of the negative and positive videos in random order.

3. Choice test: At the end of the video projection, both experimenters, wearing the same clothes as in the videos, entered the stall at the same time and placed themselves on either side of the horse, facing the horse. The assistant gave each of the experimenters the two extremities of a string passing by the halter of the horse, so that the horse was held in the middle of the stall while the assistant was leaving (Fig. S2). The experimenters held the strings at 1m from the horse and did not interact directly with her (they did not touch the horse and were looking at the ground). When the assistant had left the stall and closed the door, he gave a signal to the experimenters. At this signal, both experimenters released one extremity of the string at the same time, let the other extremity slide by the halter and wrapped the string in their hand, so that the horse was let free. The choice test then began. The test duration was 75 s, during which the experimenters tried to attract the horse for 15 s (by looking at the horse, holding out a hand towards the horse and calling it by doing a clicking sound with their mouths) and then stood still with their hands behind their back and watching the ground for 60 s. Importantly, the experimenters and the assistant did not know which videos the horse had seen before the choice test and were thus blind to which experimenter played the role of the positive or the negative experimenter.

- Control condition:

The control condition was realized with 23 new horses who were different from the ones used in the test condition. The familiarization and projection of the negative and positive videos were similar as in the test condition except that, in all videos, the horse and experimenters were replaced by moving ovals. No choice test was carried out after the video projection.

Fig. 1 Schematic representation and pictures of the video projection and the choice test

Coding and statistical analyses

The behavior of the horses was recorded with a camera during the video projection and the choice test and analyzed later using BORIS software (v. 6.0.6; Friard and Gamba 2016).

- Video projection

Four types of measures were analyzed:

1. Facial expression. Facial expressions were measured using the variables described in Lansade et al. 2018: 1) the time spent with both ears oriented backward or forward; 2) the time spent with the neck high, medium or low; and 3) the time spent with the whites of the eyes visible (as we did not observe any half-closed eyes, we did not include the time spent with the eyes wide open in the analyses since it would have been the exact opposite of the time with the

whites of the eyes visible). We only included in the analysis the variables that were expressed by the animals and visible enough on the video recordings to be analyzed without ambiguity (see Table 1 for the complete list).

2. Number of grooming attempts. The number of times the horse extended her lips to nibble the leading reins or the assistant.

3. Variation in heart rate between the beginning and end of the video. The heart rate of the horses was measured during the video projection using a heart monitor system (Polar Equine RS800CX Science, Polar Oy, Finland). The difference in mean heart rate during the first and last 5 s of each video was calculated.

4. Time spent facing the videos. We considered the horses as being attentive to the video when they directly faced the video (the angle between their head and the center of the screen was $< 15^\circ$) or when the angle was between 10° and 45° but at least one ear was oriented towards the video (Proops et al. 2009; Lampe and Andre 2012; Proops and McComb 2012; Smith et al. 2018).

- Choice test

During the choice test, we observed whether the horse touched each experimenter and how many times each experimenter was touched. Indeed, physical contacts with the muzzle has been described as an attention seeking behavior in horses (Malavasi and Huber 2016; Ringhofer and Yamamoto 2016; Trösch et al. 2019b). The touches were usually brief, but if those were longer than three seconds, it was taken into account by counting a new occurrence every three seconds (examples of this procedure: Lansade et al. 2018; Trösch et al. 2019b).

Data were analyzed by a coder who was blind to whether the video watched by the horse was positive or negative. Moreover, facial expressions and time facing the videos were analyzed by two coders since these components can be relatively ambiguous to record. The arithmetic means of the values coded by the two coders were used for the statistical analyses. Interobserver reliabilities were calculated: ears oriented forward (ICC= 0.96, lower bound= 0.94), ears oriented backward (ICC= 0.93, lower bound= 0.90); low neck (ICC= 0.93, lower bound= 0.90); medium neck (ICC= 0.98, lower bound= 0.98); high neck (ICC= 0.99, lower bound= 0.98); whites of the eyes visible (ICC= 1, lower bound= 1); time spent paying attention to the video (ICC= 0.95, lower bound= 0.92). The values obtained are all considered to represent excellent reliability (Koo and Li, 2016).

Statistics were performed with Xlstat software (Addinsoft, Paris, France) for the analysis of facial expression and with R 3.0.2 (R Core Team 2013) for all the other analyses. The significance threshold was fixed at 0.05. To cope with our limited sample size, we used nonparametric statistical tests. All of the tests we used were two-tailed. To reduce the number of two-by-two comparisons and to be able to consider facial expression as a whole (cf. Lansade et al. 2018), facial expressions were characterized using a principal component analysis (PCA) based on Spearman correlations. A Wilcoxon signed-rank test for paired samples was then performed to test whether the two first factors of the PCA of each subject differed while watching the negative and the positive videos. We used a Wilcoxon signed-rank test for paired samples to test whether the horses expressed each of the variables described above differently when watching the two videos (both in the test and the control conditions) and whether they touched one experimenter more than the other in the choice test. We used a Wilcoxon signed-rank test to test whether the variation in heart rate differed significantly from zero during the negative and positive videos. We used McNemar's test to investigate whether horses were more likely to touch one of the experimenters throughout the total duration of the test (75 s), as our data were binary. Supplementary statistical analyses comparing the results for each variable between the test and the control condition are presented in Online Resource 1.

Results

Video projection

- Test condition:

Facial expression was described by a PCA. The first factor of the PCA explained 57.30% of the total variability (Fig. 2; Table 1). It opposed two different facial expressions: “ears oriented forward”, “high neck”, and, to a lesser extent, “whites of the eyes” were negatively correlated with factor 1, while “ears oriented backwards”, “low neck” and “medium neck” were positively correlated (Table 1). The scores of the horses on factor 1 differed significantly between the two videos: horses presented significantly lower values when they watched the negative video than when they watched the positive video ($V = 33$, $P < 0.001$; Fig. 2). Factor 2 represented 16.75% of the variability and was mainly correlated with the variable “whites of the eyes” (Table 1).

The scores on factor 2 tended to differ between the two videos: the horses tended to score higher while watching the negative video than while watching the positive video ($V = 215$, $P = 0.065$).

Table 1 Correlations between the variables included in the PCA and the first two factors for the test condition

	F1 (57.30%)	F2 (16.75%)
High neck	-0.900	-0.128
Medium neck	0.822	0.051
Low neck	0.891	0.132
Ears oriented forwards	-0.742	0.177
Ears oriented backwards	0.761	0.021
Whites of the eyes	-0.164	0.968

Fig. 2 Facial expression recorded during the test condition as described by the PCA. The gray points represent the reactions towards the negative video and the black points represent the reactions towards the positive video. A Wilcoxon test showed that these two reactions differed significantly on the F1 axis. The large points represent the barycenter for each video (Negative = negative video; Positive = positive video)

The number of grooming attempts was significantly higher ($W = 131$, $P < 0.001$; Fig. 3) while watching the positive video than while watching the negative video.

The difference in mean heart rate between the first and last 5 s of the video ($W = 408$, $P < 0.001$; Fig. 3) was significantly higher for the negative video than for the positive video. This difference significantly differed from zero for both videos (negative: $V = 227$, $p < 0.001$; positive: $V = 57$, $p = 0.023$). It was positive for the negative video, meaning that the heart rate

increased while the horse watched the negative video, and was negative for the positive video, meaning that the heart rate decreased while the horse watched the positive video. The time spent facing the videos did not significantly differ between the videos ($W = 324$, $P = 0.282$).

Fig. 3 a. First factor of the PCA representing the facial expression, b. number of grooming attempts and c. difference in mean heart rate between the first and last 5 s of the video during the negative and the positive videos in the test condition. Significance was tested by a Wilcoxon test for paired samples: *** $P < 0.001$. $n = 24$ individuals

- Control condition:

The first two factors of the PCA characterizing the facial expression explained 62.79% of the total variability and are described in Table S2. None of these factors enabled significant differentiation between the two videos ($F1$: $V = 138$, $P = 0.988$; $F2$: $V = 162$, $P = 0.482$; Fig. S3). None of the other variables differed significantly between the two videos (Table S3): number of grooming attempts ($W = 241$, $P = 0.483$); variation in heart rate between the beginning and end of the video ($W = 325$, $P = 0.187$); time spent paying attention to the videos ($W = 336$, $P = 0.112$).

Choice test

The horses touched the negative experimenter significantly more frequently than they did the positive experimenter ($W = 384$, $P = 0.042$; Fig. 4). They were also more likely to touch the negative experimenter at least once when we considered the data as binary (McNemar's chi-squared = 7.11, $df = 1$, $P = 0.008$; Fig. 4).

Fig. 4 Number of touches and percentage of horses that touched each experimenter. Significance was tested by a Wilcoxon signed-rank test for paired samples for the number of touches and with a McNemar's test for the percentage of horses touching an experimenter: * $P < 0.05$, ** $P < 0.01$. $n = 24$ individuals

Discussion

In accordance with our hypothesis, our results show that the horses reacted differently when watching a positive video (with an experimenter grooming a horse) and a negative video (with an experimenter performing an unpleasant veterinary act) and that the information observed in these videos later influenced their behavior towards the two experimenters in a choice test in real life.

Horses perceived and reacted to the emotional content of the videos

In our study, the horses reacted coherently with the type of information contained in the video they were watching: they showed clear positive facial expressions during the positive video and clear negative facial expressions during the negative video; they sought contact almost exclusively during the positive videos; their heart rate decreased during the positive video while it increased during the negative video. In a control condition, we verified that these differences were not merely due to the fact that there was more movement in the negative video (as the actor horse tried to get away from the unpleasant stimulus) compared to the positive video. Indeed, in the control videos, the horse and the experimenter were replaced with moving ovals

that precisely followed their displacement and variations in surface area. The horses did not react differently when watching the two videos in this control condition. This suggests that the horses actually perceived and reacted to the content of the videos in the test condition. We can also notice that the emotional response of the horses during the control condition was intermediate between the positive and the negative video of the test condition: they did not attempt to groom the experimenter as they did during the positive video of the test and their heart rate increased less than during the negative video of the test (Online Resource 1).

Their emotional reactions while watching the videos in the test might have been caused by two different types of cues (nonexclusively). First, they might have used the emotions of the actor horse in the videos. Our results could thus be explained by an emotional contagion between the actor horse and the horse watching the video. Emotional contagion is the reflex-like trigger of one's emotion caused by observing this emotion in another individual (Hatfield et al. 1993; Boissy et al. 1998; de Waal 2007, 2012). This effect carries important functions for group living, such as the synchronization of behaviors and the consolidation of social bonds by sharing positive emotions, and reacting appropriately to an unperceived or unknown stimulus (e.g., reacting quickly to the arrival of a predator that only one member of the group has seen; Špinka 2012). Emotional contagion has been shown in various species (for a review: de Waal 2007; Špinka 2012; Briefer 2018), including farm animals: some evidence has been found among sheep (e.g., Yonezawa et al. 2017), pigs (e.g., Goumon and Špinka, 2016; Reimert et al., 2014), chickens (Edgar et al. 2011) and cattle (Boissy et al., 1998). Previous studies on horses have showed a transmission of emotions in negative contexts (e.g., Christensen et al. 2008; Keeling et al. 2009; Rørvang et al. 2015; Rørvang and Christensen 2018) but never in positive contexts. Our study suggests the emotional contagion of positive valence as well. Indeed, the horses not only reacted less negatively while watching the positive video, they also presented a facial expression associated with positive emotions (Lansade et al. 2018) and a behavioral response typical of this type of positive interaction, as they attempted to groom the assistant. Mutual grooming is an important affiliative behavior in horses (Arnold and Grassia 1982; Kimura 1998; Heitor et al. 2006) and when a human grooms a horse, the horse tends to reciprocate the grooming with humans (McGreevy et al. 2009). Second, the horses might have reacted to the valence of the experimenters' behaviors during the interaction with the actor horse (i.e., grooming the actor horse or performing unpleasant veterinary acts). Importantly, the spray used in the negative video was completely hidden inside the experimenter's hand so that the horse watching the video did not simply react to the vision of a scary object. Nor did the horses react

to the experimenters' facial expressions as we controlled both experimenters' facial expressions in the videos. In any case, our results show that the horses perceived the valence of the interactions seen in the videos and that perception induced a consistent emotional response.

Horses discriminated between the two experimenters in the choice test

During the choice test, the horses behaved differently with the experimenters based on the previously observed interaction between the experimenters and the actor horse. However, contrary to a more intuitive hypothesis, horses touched the negative experimenter more than they did the positive experimenter. One possible explanation is that the horses interpreted the negative video as a conflict between the horse and the negative experimenter. Hence, the horses might have touched the negative experimenter as an appeasement behavior. Indeed, during the postconflict periods, friendly contacts between a third-party horse and group mates previously involved in a conflict have been described in horses (Cozzi et al. 2010). Similar results have also been found in bonobos in a cooperation paradigm (Krupenye and Hare 2018). Indeed, the bonobos preferred the negative experimenter, who tried to steal a stuffed toy from the neutral experimenter, more than the positive experimenter, who helped the neutral experimenter retrieve the toy. The authors proposed that the negative experimenter might have appeared more dominant than the positive one, which would explain the bonobos' preference, as they also showed an attraction to dominant individuals in the same study. It would thus be interesting in further studies to test whether a similar phenomenon could explain the behavior of the horses towards the negative experimenter.

To discriminate between the experimenters, the horses might have adjusted their behavior towards the experimenters based on the emotional state expressed by the actor horse during the interactions seen on video: the horses eavesdropped on the emotional state of a third-party horse involved in a social interaction with a human experimenter, thereby attributing a positive or negative valence to that specific experimenter; they remembered the information and used it to adjust their later behavior towards this experimenter. This type of eavesdropping has, for instance, been shown in human infants and in dogs: they adapt their behavior with a stranger depending on their mother's/owner's emotional reaction towards the stranger (Feinman and Lewis 1983; Murray et al. 2008; Freidin et al. 2013; Duranton et al. 2016).

Furthermore, the horses might have responded to the choice test based on the experimenters' behavior seen on video, either by using the behavior in itself (e.g., the experimenters' gestures)—independently of the actor horse—or by interpreting this behavior as part of the interaction (e.g., an experimenter grooming a horse and an experimenter performing veterinary

acts). In the latter circumstance, they would have attributed a positive or negative valence to each experimenter based on the behavior the experimenter demonstrated during the interaction with the actor horse and then adjusted their own behavior in the choice test based on the experimenters' valence. This might be related to the ability of "social evaluation" described in the context of cooperation as the ability to attribute a value to a behavioral pattern demonstrated during a third-party social interaction, associate this behavior with a specific individual and use this information to adjust one's behavior towards this individual (Abdai and Miklósi, 2016). Again, the horses could not have reacted to the human facial expressions in the videos (as it was shown in Proops et al. 2018) because we made sure that both experimenters kept a neutral facial expression.

In all cases, our results suggest that horses are capable of social learning by eavesdropping on the behavior of others (McGregor 1993). This is coherent with the results of previous studies in horses that showed that they could attribute a valence to humans based on the previous interactions they had had together (Henry et al. 2005; Sankey et al. 2010). Our study goes one step further, as the horses attributed a valence based on indirect cues only—by observing a third-party interaction.

Video projection was validated as a tool to study horse behavior

The horses presented a high interest for the videos, facing them for almost the entire duration of the video projection in both the test and the control conditions (Table S3). They faced even more the videos in the test condition – showing an interaction between an experimenter and a horse- than in the control condition –showing moving ovals that had no particular meaning for them (Online Resource 1). This confirms that they perceived the content of the videos. Furthermore, they reacted accordingly to the content of the test videos: reacting negatively to the negative video and positively to the positive video. The horses also discriminated between the positive and negative experimenters they saw in the videos when meeting them for the first time in real life. This implies that the horses recognized the experimenters they saw in 2D when confronted with these experimenters in real life during the choice test. This is particularly impressive because there is a loss of information in 2D videos compared to reality (Fagot 2000); e.g., the horses did not have access to olfactory or auditory cues, nor to some visual aspects, such as depth and perspective. Previous studies have shown that horses can recognize persons they have seen in pictures (e.g., Proops et al. 2018; Stone 2009), our results show that this extends to videos as well. To recognize the experimenters, our subjects might have used the experimenters' face, body features or the color of their clothes. Hence, our findings are

particularly interesting for further studies, as video projection can be a very useful tool for behavioral studies, e.g., to study animals' understanding of emotions or of specific communication gestures.

Conclusion and implications for horse management and welfare

Our study shows that horses react appropriately to the interactions between a horse and a human seen on video: the horses expressed the same emotions as the actor horse seen in these videos via their facial expressions, behavior and heart rate. These results might be explained by a reaction to the valence of the experimenters' behavior during the interaction or by emotional contagion. This emotional contagion would have occurred by visual means only and concerned emotions of negative as well as positive valence. When meeting the real persons afterwards in a choice test, the horses discriminated between them by touching the negative experimenter more, which may be interpreted as an appeasement behavior. Our results suggest that horses are capable of perceiving the valence of an interaction seen on video, of reacting emotionally accordingly to this valence, and of social eavesdropping.

Our findings can have applied repercussions in terms of horse welfare and management. Indeed, in the case of veterinary or farriery practices that are unpleasant, it might be better to conduct them with no other horse watching; otherwise, the stress of the handled horse could spread to the other horses through emotional contagion, and the other horses might attribute a negative valence to the vet or farrier, potentially complicating future encounters with them. Finally, if horses can attribute a valence to humans after seeing a positive or negative interaction with another horse, we could also use this type of set up to test the valence of a specific interaction for horses, e.g., handling or riding methods.

Compliance with ethical standards

Disclosure of potential conflicts of interest

This study was funded by IFCE (Institut Français du Cheval et de l'Équitation; grant: Cognition-équitation).

Conflict of interest: the authors declare that they have no conflicts of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in

accordance with the ethical standards of the institution or practice at which the studies were conducted.

Acknowledgements

We thank Fabrice Reigner and his team for allowing us to use the animals and facilities and for their assistance in taking care of the horses. Our research was financially supported by the IFCE (Institut Français du Cheval et de l'Équitation).

References

- Abdai J, Miklósi Á (2016) The origin of social evaluation, social eavesdropping, reputation formation, image scoring or what you will. *Front Psychol* 7:1–13. doi: 10.3389/fpsyg.2016.01772
- Anderson JR, Kuroshima H, Takimoto A, Fujita K (2013) Third-party social evaluation of humans by monkeys. *Nat Commun* 4:1561–1565. doi: 10.1038/ncomms2495
- Aquiloni L, Gherardi F (2010) Crayfish females eavesdrop on fighting males and use smell and sight to recognize the identity of the winner. *Anim Behav* 79:265–269. doi: 10.1016/j.anbehav.2009.09.024
- Arnold, G.W., Grassia, A., 1982. Ethogram of agonistic behaviour for thoroughbred horses. *Appl. Anim. Ethol.* 8, 5–25. [https://doi.org/10.1016/0304-3762\(82\)90129-8](https://doi.org/10.1016/0304-3762(82)90129-8)
- Boissy A, Terlouw C, Le Neindre P (1998) Presence of cues from stressed conspecifics increases reactivity to aversive events in cattle: Evidence for the existence of alarm substances in urine. *Physiol Behav* 63:489–495. doi: 10.1016/S0031-9384(97)00466-6
- Bonnie KE, Earley RL (2007) Expanding the scope for social information use. *Anim Behav* 74:171–181. doi: 10.1016/j.anbehav.2006.12.009
- Briefer EF (2018) Vocal contagion of emotions in non-human animals. *Proc R Soc B Biol Sci* 285:20172783. doi: 10.1098/rspb.2017.2783
- Briefer EF, Mandel R, Maigrot AL, et al (2017) Perception of emotional valence in horse whinnies. *Front Zool* 14:1–12. doi: 10.1186/s12983-017-0193-1
- Bshary R, Grutter AS (2006) Image scoring and cooperation in a cleaner fish mutualism. *Nature* 441:975–978. doi: 10.1038/nature04755
- Cheney DL (2011) Extent and limits of cooperation in animals. *Proc Natl Acad Sci*

- 506 108:10902–10909. doi: 10.1073/pnas.1100291108
- 507 Chijiwa H, Kuroshima H, Hori Y, et al (2015) Dogs avoid people who behave negatively
- 508 to their owner: Third-party affective evaluation. *Anim Behav* 106:123–127. doi:
- 509 10.1016/j.anbehav.2015.05.018
- 510 Christensen JW, Malmkvist J, Nielsen BL, Keeling LJ (2008) Effects of a calm companion
- 511 on fear reactions in naïve test horses. *Equine Vet J* 40:46–50. doi:
- 512 10.2746/042516408X245171
- 513 Cozzi A, Sighieri C, Gazzano A, et al (2010) Post-conflict friendly reunion in a permanent
- 514 group of horses (*Equus caballus*). *Behav Processes* 85:185–190. doi:
- 515 10.1016/j.beproc.2010.07.007
- 516 Dalla Costa E, Bracci D, Dai F, et al (2017) Do Different Emotional States Affect the Horse
- 517 Grimace Scale Score? A Pilot Study. *J Equine Vet Sci* 54:114–117. doi:
- 518 10.1016/j.jevs.2017.03.221
- 519 Dalla Costa E, Minero M, Lebelt D, et al (2014) Development of the Horse Grimace Scale
- 520 (HGS) as a pain assessment tool in horses undergoing routine castration. *PLoS One*
- 521 9:1–10. doi: 10.1371/journal.pone.0092281
- 522 de Waal FBM (2012) The antiquity of empathy. *Science* 336:874–876. doi:
- 523 10.1126/science.1220999
- 524 de Waal FBM (2007) Putting the altruism back into altruism: The evolution of empathy.
- 525 *Annu Rev Psychol* 59:279–300. doi: 10.1146/annurev.psych.59.103006.093625
- 526 Duranton C, Bedossa T, Gaunet F (2016) When facing an unfamiliar person, pet dogs
- 527 present social referencing based on their owners’ direction of movement alone.
- 528 *Anim Behav* 113:147–156. doi: 10.1016/j.anbehav.2016.01.004
- 529 Earley RL, Dugatkin LA (2002) Eavesdropping on visual cues in green swordtail
- 530 (*Xiphophorus helleri*) fights: A case for networking. *Proc R Soc B Biol Sci* 269:943–
- 531 952. doi: 10.1098/rspb.2002.1973
- 532 Edgar JL, Lowe JC, Paul ES, Nicol CJ (2011) Avian maternal response to chick distress.
- 533 *Proc R Soc B Biol Sci* 278:3129–3134. doi: 10.1098/rspb.2010.2701
- 534 Fagot J (2000) Picture perception in animals. Psychology Press
- 535 Feinman S, Lewis M (1983) Social Referencing at Ten Months : A Second-Order Effect on
- 536 Infants’ Responses to Strangers. *Child Dev* 54:878–887
- 537 Freidin E, Putrino N, D’Orazio M, Bentosela M (2013) Dogs’ eavesdropping from people’s
- 538 reactions in third party interactions. *PLoS One* 8:1–8. doi:

- 10.1371/journal.pone.0079198
- Friard O, Gamba M (2016) BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol Evol* 7:1325–1330. doi: 10.1111/2041-210X.12584
- Gleerup KB, Forkman B, Lindegaard C, Andersen PH (2015) An equine pain face. *Vet Anaesth Analg* 42:103–114. doi: 10.1111/vaa.12212
- Goumon, S., Špinka, M., 2016. Emotional contagion of distress in young pigs is potentiated by previous exposure to the same stressor. *Anim. Cogn.* 19, 501–511. <https://doi.org/10.1007/s10071-015-0950-5>
- Hatfield E, Cacioppo JT, Rapson RL (1993) Emotional contagion. *Curr Dir Psychol Sci* 2:96–99. doi: 10.1111/1467-8721.ep10770953
- Heitor, F., do Mar Oom, M., Vicente, L., 2006. Social relationships in a herd of Sorraia horses. Part II. Factors affecting affiliative relationships and sexual behaviours. *Behav. Processes* 73, 231–239. <https://doi.org/10.1016/j.beproc.2006.05.005>
- Henry S, Hemery D, Richard MA, Hausberger M (2005) Human-mare relationships and behaviour of foals toward humans. *Appl Anim Behav Sci* 93:341–362. doi: 10.1016/j.applanim.2005.01.008
- Höjesjö J, Andersson P, Engman A, Johnsson JI (2007) Rapid bystander assessment of intrinsic fighting ability: behavioural and heart rate responses in rainbow trout. *Anim Behav* 74:1743–1751. doi: 10.1016/j.anbehav.2007.04.013
- Kavaliers M, Matta R, Choleris E (2017) Mate-choice copying, social information processing, and the roles of oxytocin. *Neurosci Biobehav Rev* 72:232–242. doi: 10.1016/j.neubiorev.2016.12.003
- Kawai N, Yasue M, Banno T, Ichinohe N (2014) Marmoset monkeys evaluate third-party reciprocity. *Biol Lett* 10: 20140058. doi: 10.1098/rsbl.2014.0058
- Keeling LJ, Jonare L, Lanneborn L (2009) Investigating horse-human interactions: The effect of a nervous human. *Vet J* 181:70–71. doi: 10.1016/j.tvjl.2009.03.013
- Kimura, R., 1998. Mutual grooming and preferred associate relationships in a band of free-ranging horses. *Appl. Anim. Behav. Sci.* 59, 265–276. [https://doi.org/10.1016/S0168-1591\(97\)00129-9](https://doi.org/10.1016/S0168-1591(97)00129-9)
- Koo TK, Li MY (2016) A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *J Chiropr Med* 15:155–63. doi: 10.1016/j.jcm.2016.02.012

- 572 Krueger K, Farmer K, Heinze J (2014) The effects of age, rank and neophobia on social
573 learning in horses. *Anim Cogn* 17:645–655. doi: 10.1007/s10071-013-0696-x
- 574 Krupenye C, Hare B (2018) Bonobos Prefer Individuals that Hinder Others over Those
575 that Help. *Curr Biol* 28:280–286.e5. doi: 10.1016/j.cub.2017.11.061
- 576 Lai WS, Yu WC, Liu CY, et al (2014) A new method for studying social eavesdropping
577 using male golden hamsters. *Physiol Behav* 128:202–211. doi:
578 10.1016/j.physbeh.2014.01.029
- 579 Lampe JF, Andre J (2012a) Cross-modal recognition of human individuals in domestic
580 horses (*Equus caballus*). *Anim Cogn* 15:623–630. doi: 10.1007/s10071-012-0490-1
- 581 Lansade L, Nowak R, Lainé AL, et al (2018) Facial expression and oxytocin as possible
582 markers of positive emotions in horses. *Sci Rep* 8:1–11. doi: 10.1038/s41598-018-
583 32993-z
- 584 Malavasi R, Huber L (2016) Evidence of heterospecific referential communication from
585 domestic horses (*Equus caballus*) to humans. *Anim Cogn* 19:899–909. doi:
586 10.1007/s10071-016-0987-0
- 587 Marshall-Pescini S, Passalacqua C, Ferrario A, et al (2011) Social eavesdropping in the
588 domestic dog. *Anim Behav* 81:1177–1183. doi: 10.1016/j.anbehav.2011.02.029
- 589 McGreevy PD, Oddie C, Burton FL, McLean AN (2009) The horse-human dyad: Can we
590 align horse training and handling activities with the equid social ethogram? *Vet J*
591 181:12–18. doi: 10.1016/j.tvjl.2009.03.005
- 592 McGregor PK (1993) Signalling in territorial systems: a context for individual
593 identification, ranging and eavesdropping. *Philos Trans R Soc London Ser B Biol Sci*
594 340:237–244. doi: 10.1098/rstb.1993.0063
- 595 Mennill DJ, Ratcliffe LM, Boag PT (2002) Female eavesdropping on male song contests in
596 songbirds. *Science* 296:873. doi: 10.1126/science.296.5569.873
- 597 Murray L, De Rosnay M, Pearson J, et al (2008) Intergenerational transmission of social
598 anxiety: The role of social referencing processes in infancy. *Child Dev* 79:1049–
599 1064. doi: 10.1111/j.1467-8624.2008.01175.x
- 600 Nakamura K, Takimoto-Inose A, Hasegawa T (2018) Cross-modal perception of human
601 emotion in domestic horses (*Equus caballus*). *Sci Rep* 8:1–9. doi: 10.1038/s41598-
602 018-26892-6
- 603 Nitzschner M, Melis AP, Kaminski J, Tomasello M (2012) Dogs (*Canis familiaris*) evaluate
604 humans on the basis of direct experiences only. *PLoS One* 7: e46880. doi:

- 10.1371/journal.pone.0046880
- Ophir AG, Galef BG (2004) Sexual experience can affect use of public information in mate choice. *Anim Behav* 68:1221–1227. doi: 10.1016/j.anbehav.2003.12.023
- Peake TM, Terry AMR, McGregor PK, Dabelsteen T (2001) Male great tits eavesdrop on simulated male-to-male vocal interactions. *Proc R Soc B Biol Sci* 268:1183–1187. doi: 10.1098/rspb.2001.1648
- Proops L, Grounds K, Smith AV, McComb K (2018) Animals Remember Previous Facial Expressions that Specific Humans Have Exhibited. *Curr Biol* 28:1428–1432.e4. doi: 10.1016/j.cub.2018.03.035
- Proops L, McComb K (2010) Attributing attention: the use of human-given cues by domestic horses (*Equus caballus*). *Anim Cogn* 13:197–205. doi: 10.1007/s10071-009-0257-5
- Proops L, McComb K (2012) Cross-modal individual recognition in domestic horses (*Equus caballus*) extends to familiar humans. *Proc R Soc B Biol Sci* 279:3131–3138. doi: 10.1098/rspb.2012.0626
- Proops L, McComb K, Reby D (2009) Cross-modal individual recognition in domestic horses (*Equus caballus*). *Proc Natl Acad Sci* 106:947–951. doi: 10.1073/pnas.0809127105
- Proops L, Rayner J, Taylor AM, McComb K (2013) The Responses of Young Domestic Horses to Human-Given Cues. *PLoS One* 8:26–28. doi: 10.1371/journal.pone.0067000
- Reimert, I., Bolhuis, J.E., Kemp, B., Rodenburg, T.B., 2014. Emotions on the loose: emotional contagion and the role of oxytocin in pigs. *Anim. Cogn.* 18, 517–532. <https://doi.org/10.1007/s10071-014-0820-6>
- Ringhofer M, Inoue S, Mendonça RS, et al (2017) Comparison of the social systems of primates and feral horses: data from a newly established horse research site on Serra D’Arga, northern Portugal. *Primates* 58:479–484. doi: 10.1007/s10329-017-0614-y
- Ringhofer M, Yamamoto S (2016) Domestic horses send signals to humans when they face with an unsolvable task. *Anim Cogn* 20:397–405. doi: 10.1007/s10071-017-1074-x
- Rørvang MV, Ahrendt LP, Christensen JW (2015) A trained demonstrator has a calming effect on naïve horses when crossing a novel surface. *Appl Anim Behav Sci*

- 638 171:117–120. doi: 10.1016/j.applanim.2015.08.008
- 639 Rørvang MV, Christensen JW (2018) Attenuation of fear through social transmission in
640 groups of same and differently aged horses. *Appl Anim Behav Sci* 209:41–46. doi:
641 10.1016/j.applanim.2018.10.003
- 642 Rueden CT, Schindelin J, Hiner MC, et al (2017) ImageJ2: ImageJ for the next generation
643 of scientific image data. *BMC Bioinformatics* 18:1–26. doi: 10.1186/s12859-017-
644 1934-z
- 645 Russell YI, Call J, Dunbar RIM (2008) Image scoring in great apes. *Behav Processes*
646 78:108–111. doi: 10.1016/j.beproc.2007.10.009
- 647 Sankey C, Richard-Yris MA, Henry S, et al (2010) Reinforcement as a mediator of the
648 perception of humans by horses (*Equus caballus*). *Anim Cogn* 13:753–764. doi:
649 10.1007/s10071-010-0326-9
- 650 Schindelin J, Arganda-Carreras I, Frise E, et al (2012) Fiji: An open-source platform for
651 biological-image analysis. *Nat Methods* 9:676–682. doi: 10.1038/nmeth.2019
- 652 Schuetz A, Farmer K, Krueger K (2017) Social learning across species: horses (*Equus*
653 *caballus*) learn from humans by observation. *Anim Cogn* 20:567–573. doi:
654 10.1007/s10071-016-1060-8
- 655 Smith AV, Proops L, Grounds K, et al (2016) Functionally relevant responses to human
656 facial expressions of emotion in the domestic horse (*Equus caballus*). *Biol Lett*
657 12:20150907. doi: 10.1098/rsbl.2016.0549
- 658 Smith AV, Proops L, Grounds K, et al (2018) Domestic horses (*Equus caballus*)
659 discriminate between negative and positive human nonverbal vocalisations. *Sci Rep*
660 8:1–8. doi: 10.1038/s41598-018-30777-z
- 661 Špinka M (2012) Social dimension of emotions and its implication for animal welfare.
662 *Appl Anim Behav Sci* 138:170–181. doi: 10.1016/j.applanim.2012.02.005
- 663 Stone SM (2009) Human facial discrimination in horses: Can they tell us apart? *Anim*
664 *Cogn* 13:51–61. doi: 10.1007/s10071-009-0244-x
- 665 Subiaul F, Vonk J, Okamoto-Barth S, Barth J (2008) Do chimpanzees learn reputation by
666 observation? Evidence from direct and indirect experience with generous and
667 selfish strangers. *Anim Cogn* 11:611–623. doi: 10.1007/s10071-008-0151-6
- 668 Trösch M, Cuzol F, Parias C, et al (2019a) Horses categorize human emotions cross-
669 modally based on facial expression and non-verbal vocalizations. *Animals* 9: 862-
670 872. doi: 10.3390/ani9110862

- 671 Trösch M, Ringhofer M, Yamamoto S, et al (2019b) Horses prefer to solicit a person who
672 previously observed a food-hiding process to access this food: A possible indication
673 of attentional state attribution. *Behav Processes* 166:103906. doi:
674 10.1016/j.beproc.2019.103906
- 675 Valone TJ (2007) From eavesdropping on performance to copying the behavior of
676 others: A review of public information use. *Behav Ecol Sociobiol* 62:1–14. doi:
677 10.1007/s00265-007-0439-6
- 678 Wathan J, Proops L, Grounds K, McComb K (2016) Horses discriminate between facial
679 expressions of conspecifics. *Sci Rep* 6:1–11. doi: 10.1038/srep38322
- 680 Yonezawa T, Sato K, Uchida M, et al (2017) Presence of contagious yawning in sheep.
681 *Anim Sci J* 88:195–200. doi: 10.1111/asj.12681
682
683