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1 Horses discriminate between human facial and vocal
2 expressions of sadness and joy

3

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13 Abstract

14 Communication of emotions plays a key role in intraspecific social interactions and likely in interspecific
15 interactions. Several studies have shown that animals perceive human joy and anger, but few studies
16 have examined other human emotions, such as sadness. In this study, we conducted a cross-modal
17 experiment, in which we showed 28 horses two soundless videos simultaneously, one showing a sad,
18 and one a joyful human face. These were accompanied by either a sad or joyful voice. The number of
19 horses whose first look to the video that was incongruent with the voice was longer than their first
20 look to the congruent video was higher than chance, suggesting that horses could form cross-modal
21 representations of human joy and sadness. Moreover, horses were more attentive to the videos of joy
22 and looked at them for longer, more frequently, and more rapidly than the videos of sadness. Their
23 heart rates tended to increase when they heard joy and to decrease when they heard sadness. These
24 results show that horses are able to discriminate facial and vocal expressions of joy and sadness and
25 may form cross-modal representations of these emotions; they also are more attracted to joyful faces
26 than to sad faces and seem to be more aroused by a joyful voice than a sad voice. Further studies are
27 needed to better understand how horses perceive the range of human emotions, and we propose that
28 future experiments include neutral stimuli as well as emotions with different arousal levels but a same
29 valence.

30 **Key words:** *Equus caballus*, human-animal relationship, cognition, emotion, interspecific
31 communication



32 Introduction

33 An emotion is a brief affective response to an event, which allows an individual to adapt to a change
34 in his or her environment (Désiré et al. 2002). Communicating emotions enables an individual to
35 provide information to other individuals, such that they can also adapt to the related environmental
36 change (Désiré et al. 2002; Špinka 2012). Communication of emotions can occur between individuals
37 of different species; indeed, diverse nonhuman animal species have been found to perceive human
38 emotional expressions. For example, orangutans differed in the duration of their looks toward pictures
39 of humans expressing fear or anger compared to those toward pictures of neutral faces (Pritsch et al.
40 2017), and pigeons learned to discriminate between human facial expressions of anger and joy
41 (Jitsumori and Yoshihara 1997). Studies on dogs, horses, goats and cats, focusing mainly on expressions
42 of joy and anger, have shown that these species are sensitive to human facial and vocal expressions of
43 emotions (Jardat and Lansade 2022).

44 Regarding facial expressions, dogs learned to differentiate between a smiling face and a blank
45 expression (Nagasawa et al. 2011), and they licked their mouth (a behavior usually observed in stressful
46 situations - Albuquerque et al. 2018) less when seeing a joyful face than an angry face. Dogs also
47 reacted more quickly and for longer to human facial expressions of anger or fear compared to those
48 of sadness, disgust, surprise or joy (Siniscalchi et al. 2018b). Cats spent more time close to their owner
49 and expressed more positive behaviors when they saw a person displaying joy rather than anger
50 (Galvan and Vonk 2016), and goats preferred to spend time close to pictures of humans expressing joy
51 rather than anger (Nawroth et al. 2018). In horses, a left gaze bias (generally associated with negative
52 stimuli - Siniscalchi et al. 2018b) and a quicker increase in heart rate were observed when they viewed
53 pictures of angry faces compared to joyful faces (Smith et al. 2016). When horses faced a person they
54 had previously seen smiling in a picture, they reacted differently than when they faced a person they
55 had previously seen expressing anger in a picture (Proops et al. 2018). Horses also reacted to a facial
56 expression of disgust: they followed an experimenter's gaze less in response to this expression than a
57 neutral expression (Baba et al. 2019).



58 Vocal expressions of joy also seem to provoke different reactions than those of anger in
59 domestic mammals. Horses expressed more vigilance behaviors and their heart rates increased to a
60 greater extent when they heard human vocalizations of anger compared to those of joy (Smith et al.
61 2018; Trösch et al. 2019). Cats expressed more stress-related behaviors upon hearing angry voices
62 compared to joyful voices (Quaranta et al. 2020). In dogs, a greater increase in heart rate and greater
63 reactivity were observed in response to vocalizations of anger compared to those of joy, fear or
64 sadness (Siniscalchi et al. 2018a).

65 In addition to being sensitive to human facial and vocal expressions of anger and joy, domestic
66 mammals can associate facial and vocal expressions of these emotions. In cross-modal experiments,
67 horses, dogs and cats were presented with human faces expressing anger or joy accompanied by a
68 joyful or angry voice. These animals looked differently at the pictures according to their
69 correspondence with the voice (Albuquerque et al. 2016; Nakamura et al. 2018; Quaranta et al. 2020;
70 Trösch et al. 2019), suggesting that they integrated these signals of human emotions across modalities.
71 More specifically, in experiments with dogs and cats and in an experiment with horses, a preferential
72 looking paradigm was used: two images were presented to the animals while one voice was broadcast.
73 Dogs and cats looked more at the congruent image (i.e., the one that matched the sound - Albuquerque
74 et al. 2016; Quaranta et al. 2020) whereas horses looked more at the incongruent image (i.e., the one
75 that did not match the sound -Trösch et al. 2019). In another experiment on horses, an expectancy
76 violation paradigm was used: horses saw a picture of an angry or joyful face, followed by a joyful or
77 angry voice. Horses looked longer at the speaker when the voice was incongruent with the face
78 (Nakamura et al. 2018). Thus, different responses to cross-modal stimuli were observed according to
79 the paradigm used and the species, with horses typically looking for longer durations at the
80 incongruent image (Jardat et al. 2023; Lampe and Andre 2012; Proops and McComb 2012; Trösch et al.
81 2019).



82 Overall, it is clear that horses, dogs, goats and cats are sensitive to human facial and vocal
83 expressions of anger and joy, two emotions characterized by high arousal levels (Mendl et al. 2010). It
84 remains unclear to what extent animals are sensitive to other human emotions, and if they can also
85 perceive and react to emotions with lower arousal levels. In this study, we focused on sadness, a low-
86 arousal, negative-valence emotion; the perception of human sadness by domestic mammals has been
87 described in only a few studies on dogs. Dogs approached a human more when the person was
88 pretending to cry rather than when the person was talking or humming (Custance and Mayer 2012),
89 and they seemed to express more behaviors associated with negative states when hearing vocal
90 expressions of sadness compared to those of joy (Huber et al. 2017). Moreover, viewing sad faces
91 provoked differences in heart rate variations and stress levels compared to viewing scared, joyful,
92 surprised or disgusted faces (Siniscalchi et al. 2018b). Similarly, when dogs heard sad voices,
93 differences in heart rate variations, lateralized behaviors and stress-related behaviors were observed
94 compared to when they heard voices expressing fear, anger or disgust (Siniscalchi et al. 2018a). To
95 date, it remains unclear whether horses react to human expressions of sadness. Sociological studies
96 have indicated that horse owners find emotional support in their horses, such as when feeling sad
97 (Keaveney 2008); therefore, it would be interesting to determine whether horses indeed react to this
98 emotional state.

99 We conducted a cross-modal experiment using a preferential looking paradigm. Two silent
100 videos of the same woman expressing joy and sadness were simultaneously projected while the voice
101 of another woman expressing joy or sadness was played. The purpose of the present study was to
102 examine the following research questions:

103 (1) Whether horses associate sad and joyful voices with the corresponding facial expressions.
104 We expected that horses would associate the broadcast voices with the corresponding facial
105 expressions by looking longer at the incongruent image.



106 (2) Whether horses have different behavioral and physiological reactions to human facial and
107 vocal expressions of sadness compared to joy. Horses were expected to look at the sad videos for a
108 shorter duration, and to show a lower increase in heart rate when hearing sadness compared to joy.

109 Methods

110 **Subjects**

111 The study included 36 Welsh mares (*Equus caballus*) aged 7.9 ± 2.4 years (mean \pm SD) reared at the
112 Animal Physiology Experimental Unit PAO (UEPAO, 37380 Nouzilly, France, DOI:
113 10.15454/1.55738963217 28955E12), INRAE. These mares lived in groups in indoor stalls (3-sided
114 shelter, open on one side) bedded with straw, with environmental enrichments and access to an
115 outdoor area 12h a day. Fodder and water were available ad libitum. In this facility, a general health
116 status check is performed twice a day by keepers and a more extensive clinical exam is performed at
117 least once a year by a vet. No hearing or vision issues have been reported in the horses included in this
118 study. These horses are used only for research purposes and are handled daily by humans. Thus, they
119 have the opportunity to experience human emotions including joy and sadness expressed by
120 caregivers and researchers.

121 **Experimental setup**

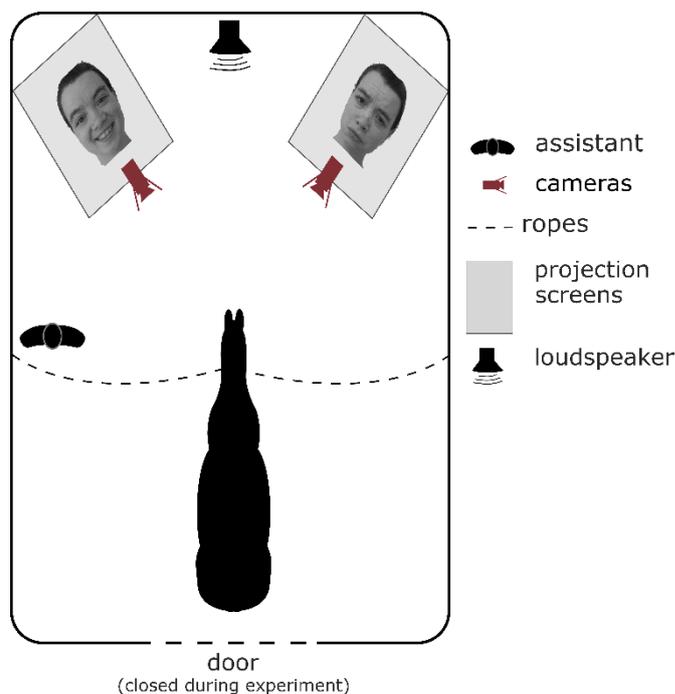
122 The experimental setup was similar to that used to investigate cross-modal recognition of human anger
123 and joy by horses as well as cross-modal recognition of adults and children (Jardat et al. 2023; Trösch
124 et al. 2019). A horse was placed in the middle of a stall and attached with two loose ropes (Fig 1).
125 Videos were projected on two projection screens (1 × 2 m) placed in front of the horse on the right
126 and left. For safety reasons, an assistant stayed with the horse to ensure that she did not panic or
127 become entangled in the ropes. The assistant stood along the wall at the level of the horse; on the
128 right of the horse for half the horses and on the left for the other half of the horses. They never
129 interacted with the horse during the tests but remained still with their head down. They were
130 instructed to stop the test if a horse panicked; no horses panicked during the experiment. The
131 experiment was filmed by two cameras, one in front of each screen. The horses were equipped with a



132 heart monitor system composed of a sensor placed on the horse and a wristwatch showing real-time
 133 heart rate values and recording beat-to-beat (RR) values (Polar Equine 149 RS800CX Science, Polar Oy,
 134 Finland). An overview camera (GoPro Hero Black) allowed the experimenter to follow the experiment
 135 from outside the stall and control the projected videos and the sounds accordingly.

Fig 1 Experimental setup.

Two simultaneous videos of a same woman expressing joy and sadness were projected while the voice of another woman speaking in a joyful or sad tone was broadcast. The videos and voices came from a validated psychological database. To respect the copyrights of this database, different images are shown here for illustrative purposes.



136 **Stimuli**

137 The videos and voices were extracted from the GEMEP Core Set database (faces: 02sad, 02joy, 07sad,
 138 07joy, 09sad and 09joy; voices: 02joy, 02sad, 06joy, 07joy, 07sad and 10joy - Bänziger et al. 2012), a
 139 validated psychological database for experimental research on emotion perception. The horses were
 140 therefore not familiar with either the faces or the voices. Pairs of videos of the same woman expressing
 141 sadness and joy were projected simultaneously, one on each projection screen, while the voice of
 142 another woman expressing sadness or joy was played over a loudspeaker placed between the
 143 projection screens (Fig 1). The faces were projected at a height of approximately 160 cm and were
 144 approximately twice the size of a real human face (as in Jardat et al. 2023; Trösch et al. 2019). The
 145 videos shown during the tests lasted 115 s and were divided into six sections of 15 s separated by 5 s
 146 intervals of black screen (Fig 2). Each section was composed of sadness and joy videos of the same



147 woman (presented simultaneously) accompanied by a voice, repeated seven times. Four different
 148 videos were generated. In each video, three women appeared twice each (once on the right side and
 149 once on the left side), in different orders. The side of the congruent video (i.e., the video that matched
 150 the emotion of the voice) was semi-randomly distributed. The side that an emotion was shown on and
 151 the emotion expressed by the voice were also semi-randomly distributed. The horses were randomly
 152 assigned to view one of the four videos, such that the order in which the women appeared and the
 153 side on which each woman expressed joy and sadness were counterbalanced among the horses.

154

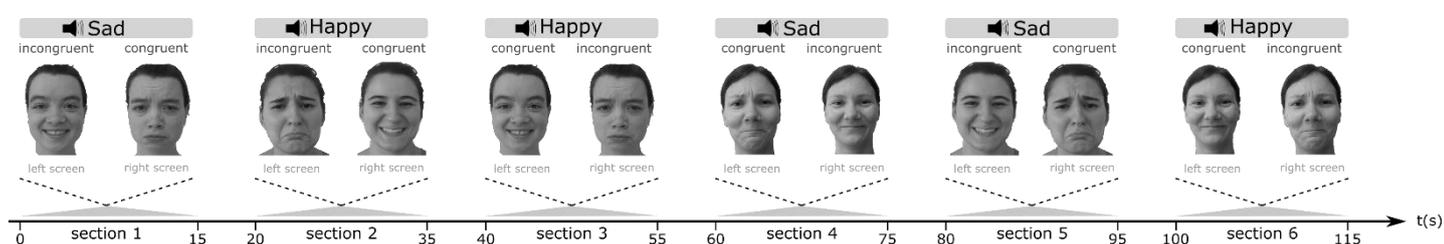


Fig 2 Example of stimuli shown to a horse. Videos of three different women expressing sadness or joy were presented, with one emotion on each side. The order in which the women appeared and the side of the sad and joyful expressions of each woman were counterbalanced among the horses. The videos and voices came from a validated psychological database. To respect the copyrights of this database, different images are shown here for illustrative purposes.

155 **Habituation phase**

156 During the habituation phase, the horse was led to the middle of the stall facing the screen. She was
 157 attached loosely to two ropes, the assistant took their position, and the habituation phase began.
 158 Identical scenes of nature were projected on both screens, while the assistant monitored the horse's
 159 heart rate on the Polar watch. If the horse moved such that her body formed an angle of less than 45°
 160 with one of the ropes, the assistant gently repositioned her to face the wall in the middle of the two
 161 projection screens. The criteria to consider that the horse was habituated to the setup were that the
 162 horse had to be calm (not neighing, pulling on the ropes, or trying to turn around and leave) and her
 163 heart rate had to have stayed below 80 bpm for two consecutive minutes (corresponding to a
 164 moderate physical effort or psychological stress – Gouyet et al. 2023; Trösch et al. 2019; Visser et al.
 165 2002; von Lewinski et al. 2013). These criteria assured that the situation was not too aversive for the



166 horse and that she was ready to pay attention to the stimuli. Once these criteria were met, the test
167 phase began immediately. If these criteria were not met after five minutes, the session ended, and a
168 new session was scheduled for the next day. Of the 36 horses initially included in the experiment, 21
169 met the criteria on day 1 and therefore continued with the test phase on the same day. Seven horses
170 met the criteria on day 2 and continued with the test phase on that day. The remaining 8 horses did
171 not meet the criteria on day 1 or day 2, indicating this setup was too aversive for these individuals.
172 Therefore, these horses were excluded from the experiment at this stage. The 28 horses that
173 successfully habituated moved forward to the test phase.

174 **Test phase**

175 Immediately after the horse met the habituation criteria, videos of women expressing sadness and joy
176 were projected (see the **Stimuli** section). The conditions were the same as those during the habituation
177 phase. If the assistant needed to reposition the horse, the experiment was paused during a black
178 screen interval and resumed once the assistant had repositioned the horse and was back in place. This
179 was a rare event that occurred only five times during the whole experiment (in one trial for two horses
180 and three trials for another horse, over a total of 168 trials for all horses). The assistant was unaware
181 of the side on which each video appeared. At the end of the test, the horse was led directly back to its
182 stall.

183 **Behavioral and physiological analyses**

184 The videos of the test phase were analyzed with BORIS software (Friard and Gamba 2016) by a first
185 coder. The videos did not contain sound and the screens were not visible in the videos, so the coder
186 was blind to the side on which each emotion appeared and the location of the congruent video. A
187 horse was considered to look at the right screen when her left eye was visible from the camera placed
188 under the right screen, and vice versa. She was considered to be attentive to the right screen when
189 her right eye could also be seen from the camera placed under the right screen (i.e., both eyes visible
190 from this camera), with both of her ears oriented forward; and vice versa. For each section, the total



191 looking duration and duration of being attentive to each video, the number of looks and the duration
192 of the first look toward each video, and the latency between the start of a section and the first look
193 toward each video were quantified. Thirty percent of the videos (9 individuals) were analyzed again by
194 a second coder to evaluate scoring reliability. Interclass correlation coefficients (ICC) and their 95%
195 confidence intervals were calculated and appreciated according to Koo and Li's method (2016),
196 showing good to excellent reliability for three variables and moderate variability for two variables
197 (duration of being attentive: 0.91[0.86,0.93] – excellent, looking duration: 0.82[0.75,0.88] – good,
198 latency of the first look: 0.83[0.76,0.88] – good, duration of the first look: 0.70[0.59,0.78] – moderate,
199 number of looks: 0.62[0.50,0.73] – moderate). As all the variables showed a same trend (all differed
200 significantly according to the emotion, in the same direction – see **Results**), we decided to keep them
201 all in the statistical analysis.

202 RR data were extracted from the Polar recordings. A visual correction was applied to eliminate
203 artifactual beats (as recommended by von Borell et al. 2007, see Supplementary Information (SI) – Fig
204 S1). RR values were converted to heart rate values and the difference in heart rate (in beats per
205 minute—bpm) between the last and first 5 s of each section was calculated. Data were missing for
206 three individuals due to technical issues with the heart rate monitor. Therefore, the heart-rate analysis
207 included data from 25 individuals.

208 **Statistical analyses**

209 All statistical analyses were performed using R 4.1.2 (R Core Team 2021), and figures were created
210 using the packages *ggplot2* (Wickham 2016), *ggpubr* (Kassambara 2020) and *survminer* (Kassambara
211 et al. 2021). Significant differences were considered at $p \leq 0.05$.

212 *Behavioral analysis*

213 The durations of looking at and of being attentive to each video, the number of looks, and the
214 duration of the first look toward each video were analyzed according to the congruence of the video
215 with generalized linear mixed models (GLMMs) from the package *glmmTMB* (Brooks et al. 2017), using



216 linear, Tweedie and Poisson distributions as appropriate for the given variable (see Table 1). The
 217 latency to look at the video was investigated according to the congruence of the video with the sound,
 218 and to the emotion displayed in the video, using survival analyses from the package *coxme* (Therneau
 219 2022). This allowed us to include in the analysis horses that did not look at one of the videos at all
 220 during a section.

221 For each of the five response variables, an initial model was constructed to assess the
 222 interaction effect of video-sound congruence with the emotion displayed in the video. The side of the
 223 watched video was added to control for potential side biases. Horse identity was added as a random
 224 effect to account for individual variation in paired data, as each horse was tested six times.
 225 Distributions, within-group variance and homoscedasticity of the residuals for the GLMM were
 226 checked using the package *DHARMA* (Hartig 2021). For the survival analysis, outliers, the linear
 227 assumption and the proportionality assumption were evaluated graphically. If a horse did not look at
 228 one of the videos during the test, the latency to look at this video was set as censored (this happened
 229 56 times). The best version of each model was selected according to model comparison via two-tailed
 230 ANOVA with the null model and simpler models (without the interaction, then without each variable
 231 of interest). The selected models are presented in Table 1 (see SI - Table S1 for the detailed results of
 232 each ANOVA).

233 **Table 1: Results of the model selections for each variable.** Detailed results of ANOVA for the
 234 selection of each model are provided in SI - Table S1.

Response variable (y)	Model type	Family	Selected formula	X ²	DF	p value
Duration attentive to video	GLMM N=28	Tweedie	y ~ congruence + emotion	27.0	1	<0.001***
Duration looking at video	×6 sections ×2 sides	Gaussian	y ~ congruence + emotion	21.6	1	<0.001***
Duration of first look		Tweedie	y ~ congruence + emotion	13.9	1	<0.001***
Number of looks		Poisson	y ~ congruence + emotion	6.9	1	0.009**
Latency of first look	Coxme N=28×6 sections×2 sides Censored = 56		(y, censor) ~ congruence + emotion	7.0	1	0.008**
Heart rate variations	GLMM N=28 ×6 sections	Generalized Poisson	y ~ emotion	2.88	1	0.09 °



235

236 Additionally, outliers were identified using the boxplot method and the GLMM analyses were
237 repeated (Aguinis et al. 2013), showing they did not influence the results (see SI – Table S2). Moreover,
238 the influence of the experimenter manipulations on a few horses (see Methods) was checked,
239 revealing it did not influence the results (see SI – Table S3).

240 The duration of the first look toward each video was also analyzed using a binomial
241 transformation. For each horse, the variable was set at 1 if the first look (in the first section) toward
242 the incongruent video was longer in duration than the first look (in the first section) toward the
243 congruent video, and was set at 0 if it was not. The number of horses that looked more at the
244 incongruent video was compared to chance using a one-tailed binomial test (*binom.test* with $p=0.5$
245 and $n=28$).

246 *Heart-rate analysis*

247 Heart-rate variation was investigated with GLMMs from the package *glmmTMB* (Brooks et al. 2017),
248 using a generalized Poisson distribution (*family=genpois*). A model was constructed to assess the effect
249 of the voice heard. Horse identity was added as a random effect to account for individual variation in
250 paired data, as each horse was tested six times. Distributions, within-group variance and
251 homoscedasticity of the residuals were checked using the package *DHARMA* (Hartig 2021). The model
252 was selected according to model comparison via two-tailed ANOVA with the null model (Table 1, see
253 SI - Table S4 for the detailed results of the ANOVA).

254 Results

255 Binomial analysis revealed that the number of horses whose first look toward the incongruent video
256 was longer than the first look toward the congruent video was higher than expected by chance (Fig 3,
257 one-tailed binomial test, $p=0.04$).



258 This effect was absent when analyzing horse behaviors from the whole experiment.
259 Generalized linear mixed models (GLMMs) and survival analyses did not reveal a significant influence
260 of the video congruence with the sound on the attention and looking behavior of horses (duration
261 spent being attentive: $Z=0.4$, $p=0.6$; looking duration: $Z=0.6$, $p=0.5$; duration of the first look: $Z=1.3$,
262 $p=0.2$, number of looks: -0.3 , $p=0.7$; latency to look: $Z=-0.2$, $p=0.9$).

263 GLMMs revealed that the time horses spent being attentive to the video, the duration looking
264 at the video, the duration of their first look, the number of looks and the latency to look at the video
265 were all significantly influenced by the emotion displayed by the humans in the video (selected models
266 are indicated in Table 1). Horses were more attentive, looked for a longer duration, had a longer first
267 look and a higher number of looks toward the joyful video compared to the sad video (Fig 4a-d,
268 duration being attentive: $Z=-5.23$, $p<0.001$; duration spent looking, $Z=-4.72$, $p<0.001$; duration of the
269 first look: $Z=-3.75$, $p<0.001$, number of looks: -2.61 , $p=0.009$, see SI - Table S5 for model estimates).
270 Survival analysis revealed that horses looked significantly faster to the joyful video than the sad video
271 (Fig 4e, latency to look, $Z=-2.65$, $p=0.008$).

272 The side of the watched video was never included in the selected models (i.e., the models
273 selected after applying the selection criteria), which indicated that the side of the watched video did
274 not explain the variation in the response variables and that horses did not exhibit a side bias (Table 1,
275 SI - Table S1).

276 The variation in heart rate approached significance and tended to be higher when hearing
277 joyful voices than when hearing sad voices (Fig S2, $Z=-1.7$, $p=0.09$, see SI - Table S3 for model
278 estimates).

279

280



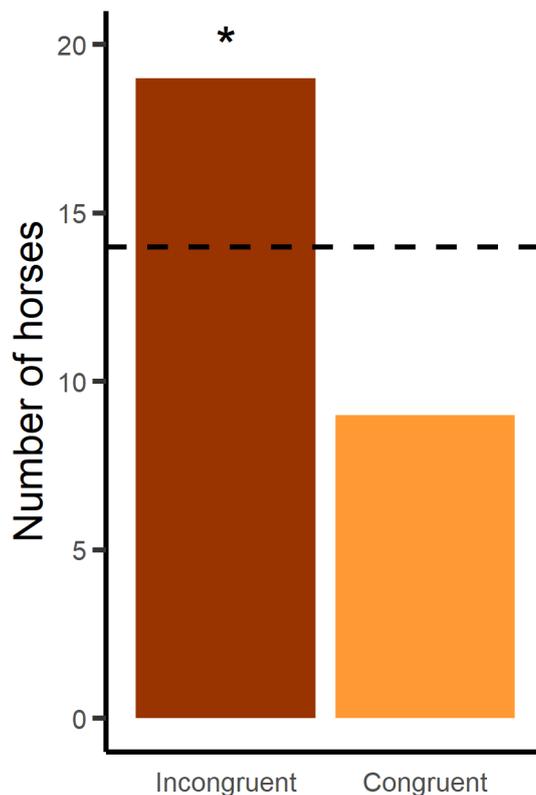


Fig 3 Horses' first look was longer toward the incongruent video. The number of horses whose first look toward the incongruent video was longer than their first look toward the congruent video (19 horses) was higher than chance (half of the horses, represented by the dashed line). *: $p \leq 0.05$, unilateral binomial test, $n=28$.

281 Discussion

282 In this study, horses seemed to associate voices expressing sadness or joy with the corresponding facial
 283 expressions, at least in their first looks toward the videos. Indeed, more horses than expected by
 284 chance exhibited a longer duration of the first look toward the incongruent video than the first look
 285 toward the congruent video. Moreover, horses had a lower latency to look, looked a higher number of
 286 times and looked for longer durations at the videos of joyful faces than those of sad faces. Horses also
 287 showed different physiological reactions to the human voices expressing emotions, with heart-rate
 288 variations tending to differ when they heard joyful voices compared to sad voices.

289 The significant number of horses whose first look toward the incongruent video was longer
 290 than their first look toward the congruent video is consistent with the findings of previous cross-modal
 291 experiments in horses (Jardat et al. 2023; Nakamura et al. 2018; Trösch et al. 2019). In other words,
 292 horses' first look toward the videos was preferentially directed toward the video that did not
 293 correspond to the sound heard. This finding suggests that when first hearing the voice, horses were



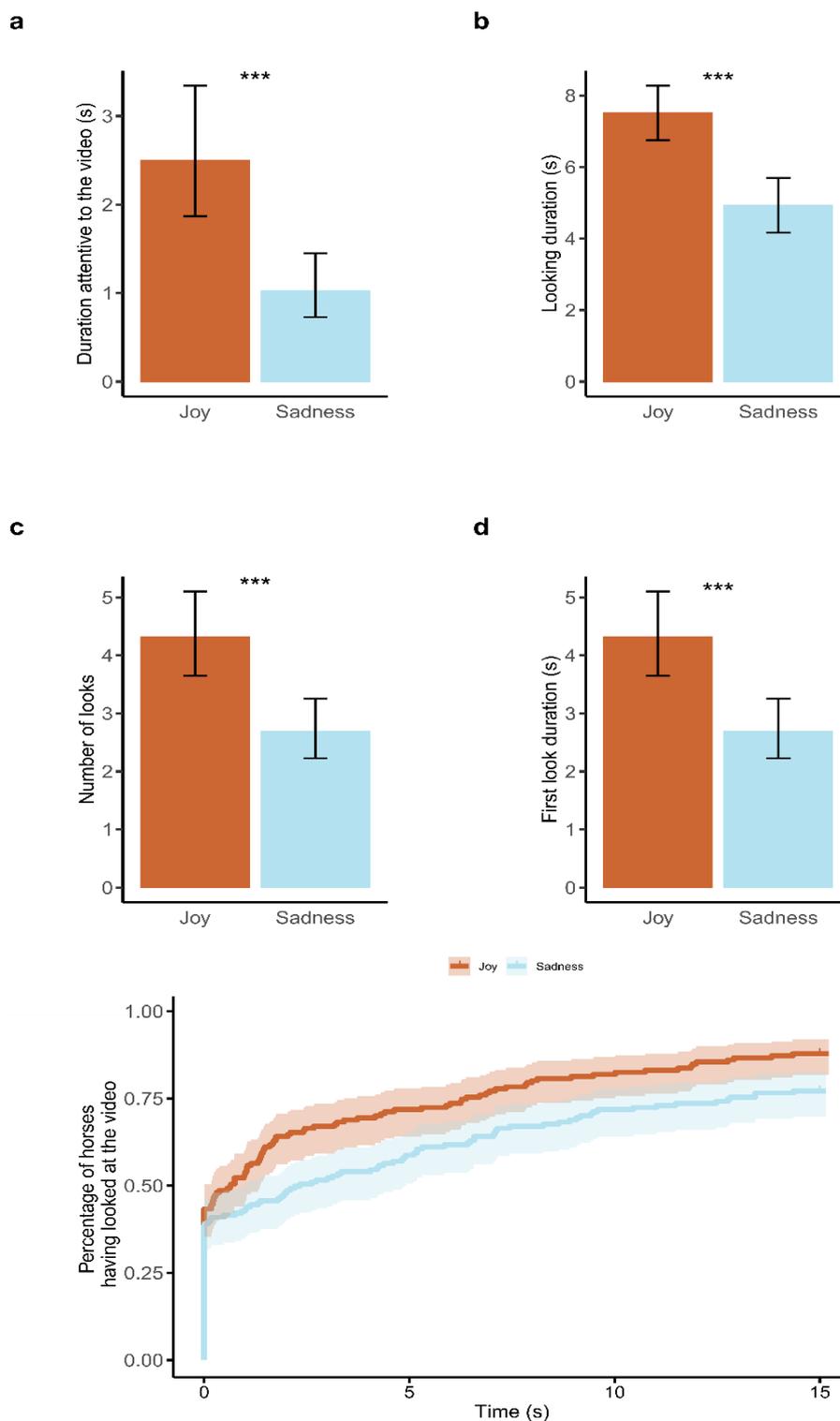


Fig 4 Horses looked more at the joyful video. Attention and looking behavior of horses toward the joy and sadness videos. (a) Duration attentive to each video during a video section. (b) Looking duration to each video. (c) Number of looks toward each video. (d) Duration of the first look toward each video. (e) Survival analysis for the latency of the first looks toward each video. The figures were generated from the corresponding models presented in Table 1. The error bars represent the standard errors from the models. ***: $p \leq 0.001$.

295 surprised by the incongruency with the observed emotional face, indicating that they
296 associated the facial and vocal stimuli that expressed the same emotion. This result may indicate that
297 horses form cross-modal representations of joy and sadness in which vocal and facial features are
298 associated (Albuquerque et al. 2016; Jardat et al. 2023; Quaranta et al. 2020). This preference for the
299 incongruent video was not observed further in the test, which could be explained by horses' strong
300 preference for the joyful face after first appraising both videos, as we discuss below.

301 Horses looking behavior toward the videos showed that they looked faster and for a longer
302 duration at videos displaying joy than sadness. Other experiments with domestic mammals have
303 shown that horses, dogs, cats and goats react more positively (e.g., longer looks and less stress-related
304 behaviors) to human facial expressions of joy compared to those of anger (Albuquerque et al. 2018;
305 Galvan and Vonk 2016; Nawroth et al. 2018; Smith et al. 2016, 2018). On the physiological level, we
306 measured heart rates to assess arousal (Briefer 2018; Jardat et al. 2022). A change in heart rate can be
307 attributed either to an increased stimulation from the sympathetic system or a decreased stimulation
308 from the parasympathetic system, which are both affected by the arousal level of the horse (Patteson
309 1996). Thus, the differences in heart rate variations according to the emotion heard suggest that the
310 arousal level of horses tended to increase when they heard joy, a high-arousal emotion, and to
311 decrease when they heard sadness, a low-arousal emotion (Mendl et al. 2010). This suggests that the
312 arousal levels of horses can be influenced by the arousal levels of human expressions they hear.
313 Similarly, previous studies reported higher or increasing heart rates in horses upon seeing or hearing
314 expressions of anger compared to those of joy (Siniscalchi et al. 2018a; Smith et al. 2016, 2018; Trösch
315 et al. 2019).

316 Horses' longer, faster and more frequent looks toward faces expressing joy compared to those
317 expressing sadness could be due to at least three mechanisms. (1) The most parsimonious hypothesis
318 is that horses reacted to the visual and acoustic properties of the stimuli: they may have been more
319 attracted to the joy videos because of the greater movement in these videos, and they may have been



320 more aroused by the joyful voices because of the higher pitch and greater pitch variations in these
321 recordings. (2) Alternatively, horse reactions could stem from associations formed by horses during
322 interactions with humans; horses may have observed humans expressing joy in more positive and
323 arousing contexts compared to the contexts in which they observed humans expressing sadness. (3)
324 Finally, emotional contagion could take place between humans and horses; that is, horses could match
325 the perceived emotional state of humans in an unconditioned way (Pérez-Manrique and Gomila 2022).
326 Joy is a more positive and higher arousal emotion than sadness (Kremer et al. 2020; Mendl et al. 2010);
327 thus, a joyful face may induce more positive emotions in horses, and horses could prefer to look at
328 joyful faces compared to sad faces. However, it is not known whether horses experience sadness
329 themselves. We are unable to evaluate the subjective feelings (e.g., affect) of animals; moreover
330 sadness is particularly seldom cited as an emotion that animals likely experience, in contrast to other
331 emotions such as fear (or an alarmed state), that have a clearer link to adaptative benefits of avoiding
332 sources of danger (e.g., Corujo et al. 2021; Désiré et al. 2002; Hall et al. 2018; Kremer et al. 2020; Špinka
333 2012). Sadness was not discussed in foundational texts on animal emotions (e.g., Darwin 1872), and
334 current studies on emotional states of horses do not list low-arousal negative-valence states (Corujo
335 et al. 2021). This may be explained by the difficulty of detecting sadness in animals. Indeed, low-arousal
336 states are more difficult to characterize than high-arousal states (Kremer et al. 2020), and among low-
337 arousal states, differentiating positive and negative valences can be challenging (Fureix and Meagher
338 2015). Moreover, for prey animals such as horses, expressing emotions suggestive of vulnerability,
339 such as sadness, can be disadvantageous (Hall et al. 2018). However, sadness or low-arousal negative-
340 valence states such as boredom, anhedonia or inactivity have been described in studies of owner and
341 caretaker perceptions of equine emotional states (Hötzel et al. 2019) as well as studies based on
342 behavioral observations (Fureix et al. 2015; Fureix and Meagher 2015), e.g., of horses housed in
343 individual boxes (Ruet et al. 2019). In addition, the proposed adaptative role of sadness to encourage
344 the formation of social bounds (Panksepp 1998) could be relevant for social species such as horses
345 (VanDierendonck and Goodwin 2005). Thus, it is possible that horses experience emotional states



346 related to sadness. The potential recognition of human sadness by horses could, moreover, contribute
347 to the human-horse relationship by facilitating interactions and the formation of social bonds.

348 This study has several limitations and opens up new research avenues. First, there was no
349 neutral control stimuli in this study, which limits our conclusions to comparisons between joy and
350 sadness. In future studies, comparing horses' reactions to human expressions of joy or sadness to their
351 reaction to neutral expressions could help further describe how horses perceive each of these
352 emotional states in humans. It would also be interesting to investigate whether horses can discriminate
353 human emotional states with different arousal levels but a same valence, for example a joyful state
354 (positive, high-arousal) compared to a calm/happy state (positive, low-arousal). Indeed, both our
355 results and previous studies on anger and joy (Proops et al. 2018; Smith et al. 2018, 2016) indicate a
356 preference for happy (positive, high-arousal) expressions; and understanding whether horses also
357 discriminate between a joyful and a calm/happy state in humans could be helpful for both welfare and
358 practical applications by riders and handlers. Last, further studies could explore the possible emotional
359 contagion of human-expressed sadness to horses. In response to human expressions of sadness,
360 horses' emotional valence as well as changes in behavior, especially during human-horse interactions,
361 could be evaluated (Adriaense et al. 2020).

362 In conclusion, in this study, we showed that horses seem to associate human vocal and facial
363 expressions of sadness and joy cross-modally. Moreover, horses were more attracted to facial
364 expressions of joy than sadness and they seemed to be more aroused by human voices expressing joy
365 than those expressing sadness. These findings indicate that horse owners, riders and caretakers could
366 benefit from expressing joy when interacting with horses. The related mechanism could be emotional
367 contagion from humans to horses, a phenomenon often regarded as a premise of empathy (Adriaense
368 et al. 2020).



369 Declarations

370 Ethical Approval

371 This study was approved by the Val-de-Loire ethical committee – authorization number CE19 2022-
372 1003. French and European guidelines for the care and use of animals were followed.

373 Competing Interests

374 The authors have no competing interests to declare that are relevant to the content of this article.

375 Authors' contributions

376 All authors devised the protocol. PJ, CP, FR, and LL implemented the protocol. PJ, CP, and LL coded
377 the videos and analyzed the heart-rate and behavioral data. PJ, OL and LL performed the statistical
378 analysis. PJ, OL, LC, and LL revised the analysis and report.

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382 Availability of data and materials

383 Data and code generated and analyzed in this study have been deposited at
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391

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