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An inconsistent social buffering effect from a static visual substitute in horses (*Equus caballus*): A pilot study

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ABSTRACT

Social buffering occurs when a companion helps to reduce a subject's stress response during a stressful event and/or to recover more quickly from a stressful event. The aim of this study was to determine if any social buffering function of a horse companion can occur in relation to a visual substitute (poster of a relaxed horse face) during two different stressful situations: a novel object test (a ball presented gradually) and an umbrella test (sudden opening of an umbrella). In order to evaluate the effect of a horse face poster on the subject's stress responses, behavioral (reactivity score) and physiological (heart rate) measurements were taken. Each of the 28 study subjects completed four trials: two novel object tests and two umbrella tests—each with a relaxed horse face poster and a pixelated poster (control). Our results showed that the presence of a horse face poster might significantly reduce the behavioral response (reactivity) of subject horses in the novel object test but did not improve heart rate recovery (HRR) time. The horse face poster did not have a significant effect on the behavioral reaction or HRR in the umbrella test. Any effect of a horse face poster as a potential social buffer might potentially depend on the nature of the stressful event.

Introduction

Social buffering occurs when a companion helps to reduce the subject's stress response during a stressful event and/or speeds recovery from a stressful event (Cohen and Wills, 1985; reviewed by Kikusui et al. [2006], Hennessy et al. [2009], and Rault [2012]). For example, the presence of the mother when chicks received an air puff (aversive stimulus) helped the chicks to reduce their stress response (Edgar et al., 2015). However, in the absence of the physical presence of a companion, social buffering might still occur. A few studies in a limited range of species have shown that the companion can be substituted by the presence of specific sensory stimuli (marmoset: Rukstalis and French [2005], rat: Takahashi et al. [2013], and sheep: da Costa et al. [2004]). For example, in marmosets, during short-term social isolation from their familiar pair mates, subjects exposed to vocalization from their pair mates showed significantly lower levels of physiological stress (urinary cortisol) (Rukstalis and French, 2005). These primates possess a rich vocal repertoire and their vocalizations have a signature, thus making it possible to distinguish each member within the group (Jones et al., 1993; Jorgensen and French, 1998; Smith et al., 2009). Social buffering based on olfactory cues also has the potential to replace the presence of an individual. For example, in rats, a subject exposed to a conditioned stimulus in a box that has the odor of a conspecific may show a reduction in freezing behavior and changes in neural activity, especially the Fos expression (expression of the gene c-fos is high during stressful situations) in the paraventricular nucleus compared to those exposed to a clean box (Takahashi et al., 2013). However, a stressed companion may release alarm pheromones (Abel, 1991; Inagaki et al., 2014), which can increase the anxiety of other rats (Inagaki et al., 2014) and consequently undermine a potential social buffering effect. A lesion of the main olfactory epithelium of the subject rat blocks the latter social buffering effect (Kiyokawa et al., 2009), reiterating the importance of olfactory information to that species. Indeed, it is essential that any potential substitute social cue is relevant for the species studied in order to maximize the chances of any social buffering effect.

Few studies have used a visual substitute to replace the companion despite its importance in discrimination and potentially recognition in many species (e.g., sheep; Kendrick et al., 1995, 2001). Ungulates have shown to be able to relate a 2D image to a conspecific (cattle: head images, Coulon et al. [2009]; horse: whole-

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body images, Grzimek [1943]; and sheep: whole-body images, Vandenheede and Bouissou [1994]). Da Costa et al. (2004) found that a picture of a conspecific sheep reduced behavioral (locomotor activity and protest vocalizations) and physiological (heart rate, cortisol, and adrenaline) responses during social isolation. In horses, Mills and Riezebos (2005) found that stereotypic weaving was reduced over a 2-day period when subjects were exposed to a life-size poster of a horse's head. However, in cattle, it has been noted that a visual substitute, in the form of a conspecific facial image, becomes less efficient after 2 h of social isolation (Ninomiya and Sato, 2011). In their study conducted on seven mares, Rogers et al. (2012) used a static image during the separation of mare and foal. In this study, they used two different visual stimuli: a "foal phantom" (black image of a foal with curious and alert head posture), a square shape (black square), and a control situation (no visual stimuli). The foal phantom helped to reduce the mares' behavioral and physiological (heart rate) responses compared to the square shape or control situation. However, the authors note that they were not sure that the mare did not also receive olfactory signals from her foal, as the foal was just 3-4 m away (not visible to her). Recently, videos have been used to study potential emotional contagion in horses (Trösch et al., 2020), which may be relevant to at least some social buffering effects. Emotional contagion can be defined as the tendency of an individual to automatically synchronize and mimic expressions such as facial, postural, vocal, and whole-body movements with those of another individual's. Consequently, the two individuals converge emotionally (Hatfield et al., 1993, 2011). In the study of Trösch and colleagues (2020), horses were exposed to two short videos without sound (30 s each): one showing typical horse facial expressions of positive emotions with a human grooming it; the other involving negative emotions from a human performing a veterinary act on it (applying a spray toward the horse's head, but with the spray not visible on the video). The two videos had a strong effect on both the behavior and the physiology of the viewing subject. The researchers suggest that these differences arise because the horse can perceive the emotions presented in the video, and it is possible that there is an emotional contagion between the subject and the horse in the video.

The horse is a good model for research on social buffering as it is a gregarious species with a complex social system (Feh, 2005; Cozzi et al., 2010). Horses can communicate through subtle visual signals such as facial expressions, eye direction, and ears position (Wathan and McComb, 2014; Wathan et al., 2015; reviewed by Fureix et al. [2012]) and adapt their behavioral response according to the facial expression of horse pictures (e.g., positive attention, relaxed, agoniztic) (Wathan et al., 2016). During a sudden stressful situation involving the sudden rise of a 1 kg black plastic bag, the presence of a habituated companion can help to reduce the fear reactions of unrelated young horses (Christensen et al., 2008). Our previous research (Ricci-Bonot et al., 2021) has examined social buffering with a live conspecific in both a novel object test (gradual presentation of a ball) and an umbrella opening test (sudden opening of an umbrella). Our results highlighted that social buffering can be significantly influenced by the nature of the stimulus presented, but, in horses, does not seem to be affected by companion's habituation status or familiarity. The type of stimulus used as a stressor also produced differential effects on behavioral and physiological measures of buffering. A companion significantly reduced the subject's behavioral response (reactivity) in the novel object test but not in the umbrella test. However, heart rate recovered more quickly for subjects with a companion in the umbrella test but not in the novel object test. We proposed that circumstances which allow greater control over the environment and processing of the nature of the novelty facilitate the demonstration of behavioral effects of social buffering. In contrast, startling stimuli do not allow this higher level processing, and in this case, any effects of social buffering are likely to be manifest only in measures of physiological recovery, if at all. Previous studies on the use of visual social cues in other ungulate species (cattle: Ninomiya and Sato [2011]; sheep: da Costa et al. [2004]) have focused only on social isolation as the stressor. The aim of this study was therefore to determine if the social buffering function of a companion could be replaced by a visual substitute during situations involving physical stressors: a relatively static novel object and a more dynamic umbrella opening test.

Material and methods

The University of Lincoln Research Ethics Committee approved this research (CosREC433) and all methods were carried out in accordance with the ethical guidelines of the International Society for Applied Ethology (Sherwin et al., 2003).

Animals

The study took place in "Les Ecuries de Pehou" (Plouër-sur-Rance, France) from January to February 2020 (6 weeks); a facility for privately owned and commercially produced horses on a 10-hectare site.

A total of 28 horses, 18 males (geldings) and 10 females, aged 4-16 (M = 8.5, SD = 3), of five different breeds (Anglo Arab, Belgian Warmblood, French saddle, Zangersheide, and indeterminate breed) were used in these studies. These horses were all in training and were individually housed. The pictures of four horses, two males (geldings) and two females, aged 5-28 (M = 14.25, SD = 9.88), of four different breeds (British Warmblood, Irish Draught cross, KWPN Warmblood, and unknown breed) were used: three horses for the creation of a database and one horse for the visual substitute stimulus.

Materials

Suitable potential visual substitute for social buffering

As noted by Mills and Riezebos (2005), the expression of a horse's face in a poster may affect the viewer's behavior and level of arousal. To maximize any potential social buffering effect, in the current study, it was considered important to use an image of a relaxed horse. To determine the characteristics of a relaxed posture in horses, three horses were photographed in different attitudes to create a database of horse faces. This consisted of nine pictures representing three horse faces (only the head) in three different positions and was sent to four Ph.D.-qualified researchers with published peer-reviewed papers on equine behavior. They rated the images from the least to the most clearly relaxed and explained their choices. The researchers based their decisions on different regions of the head (ears, eyes, nose, mouth, and head elevation). All four researchers cited the position of the ears as a key factor, three cited the eyes and the head elevation, and two cited the nose (nostril) and mouth (lips).

These criteria were then used to choose a photograph of a fourth horse to represent a clearly relaxed horse for this experiment. In this photograph, which was chosen from a new poll of photographs using the criteria suggested by the experts, but not sent for evaluation, the horse's head was a left lateral view (Figure 1a). The horse used in the photograph was unfamiliar to all the subjects used in the experiment. The background of this poster was black to reduce extraneous variation and allow focusing on the horse's head only.

A pixelated poster, consisting of 54 pieces cutout from the horse poster and randomly rearranged (as per Mills and Riezebos, 2005) was used as a control treatment (Figure 1b). This allowed the same visual input but without the horse stimulus.

Experimental setup

A rectangular (9 m x 3 m) test arena was built with wooden poles with a starting area (3 m x 3 m) (Figure 2a). The poles were

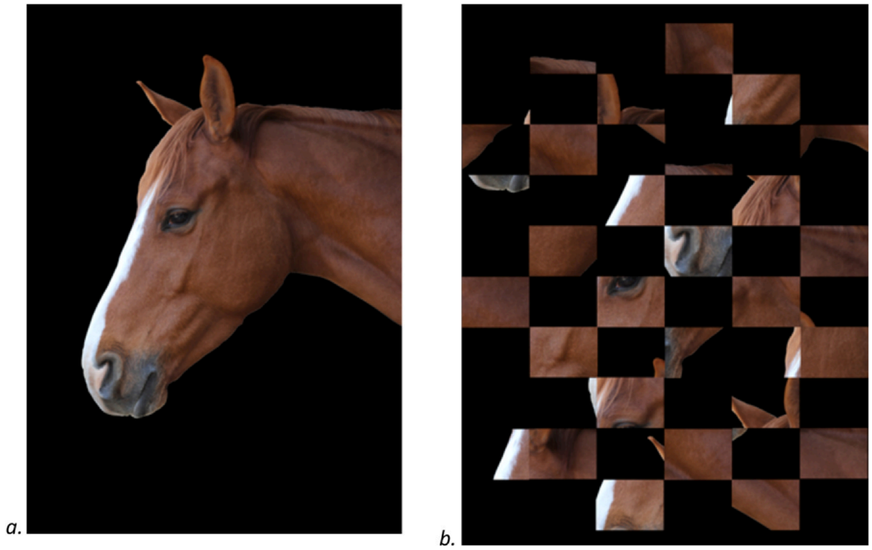

Figure 1. images used for horse face poster (a) and pixelated control poster (b).

placed at a height of 1 m from the ground with nothing between the pole and the ground. The testing subject was brought into this starting area, held until the arena was closed and then the rope detached from the head collar.

The test arena contained one food bucket in order to orientate all subjects in the same direction and at the same distance from both the stimulus (novel or dynamic stimulus) and the poster (horse face or control poster). The distance between the different stimuli and the food bucket was 1 m.

The potential visual substitute (life-size image of a horse face) or the visual control (pixelated image) was on the right side of the arena, at a distance of 1.60 m from the horse so the horse could touch it if it wanted to. Wooden boards were placed under the poster so the image was not simply floating in midair (Figure 2b).

The poster (594 mm by 841 mm) was made of a mat monomeric laminate on a 3 mm Dibond sheet and had a resolution of 7016 x 9933 pixels.

Test stimulus

The two stressors used were the same as per Ricci-Bonot et al. (2021): the gradual introduction of a novel 75 cm black and white striped ball (novel object test) and a novel dynamic stimulus, the sudden opening of a blue and white 120 cm umbrella (umbrella test).

Methods

General habituation to the test environment

The horses were habituated individually to the test arena before the beginning of the experiment to reduce the risk of stress due to a novel environment by using the same principles of habituation described by Christensen et al. (2008). The horses were considered habituated and ready to participate in the experiment when they entered the arena voluntarily and walked directly to the bucket to eat for at least 90 s out of a total of 120 s. During this habituation process, the closed umbrella was present and the person who would open the umbrella stood still behind it to habituate the horse to their presence. The horse face poster or pixelated face poster was replaced by a black poster during this habituation phase, in order to reduce the risk of habituation to the treatment intervention.

Experimental procedure

The heart rate monitor (Polar Equine H7) was strapped to the horse's chest in the stable before bringing the horse to the test arena.

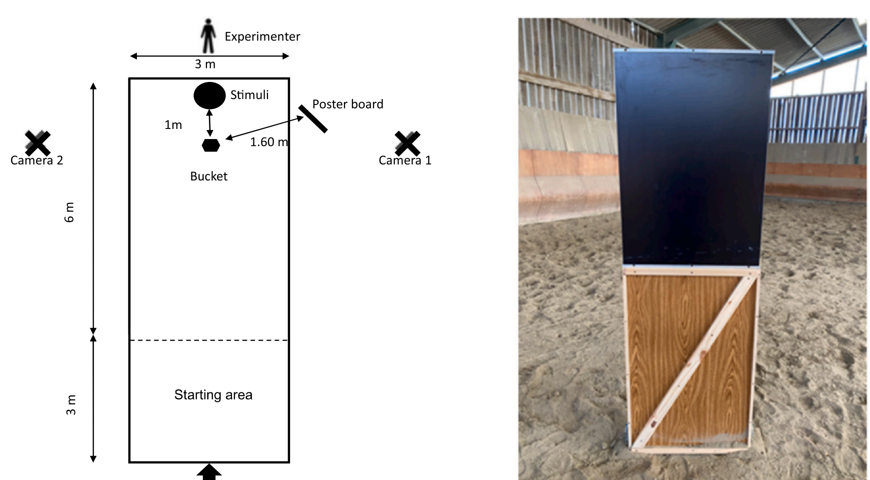

Figure 2. (a) Test arena for novel object and umbrella test (dotted line = line drawn in the sand) and (b) mounting for the visual substitute.

Figure 3. Subject during the umbrella test with the pixelated poster.

Once the horse was in the starting area, the test arena was closed and the rope detached from the horse, who was free to move in the test arena. As in our previous studies, the novel object was introduced gradually into the test arena by an experimenter who passed the ball under the pole while remaining outside the arena, or the umbrella was opened, when the horse had eaten with its head in the bucket for 3 s (Ricci-Bonot et al., 2021). From this moment, the test ran for 5 min. For the umbrella test, the umbrella was kept open for the 5 min of the test and its tip was one meter from the food container (Figures 2a and 3). At the end of the 5 min, the horse was brought back to its stable, and the heart rate monitor was removed.

Each horse performed four tests (one per week): (I) novel stimulus with horse face poster, (II) novel stimulus with pixelated poster, (III) sudden stimulus with horse face poster, and (IV) sudden stimulus with pixelated poster. The horses were randomly allocated to four groups; each group performed the tests in a different order to reduce possible order effects between subjects (see Table 2). Following randomizing of the order, three of the four test groups received the pixelated poster treatment before the horse face poster treatment.

Table 2
Order of tests for each group.

	NO-PI	NO-HP	UM-PI	UM-HP
Group 1 (seven horses)	1	2	3	4
Group 2 (seven horses)	4	1	2	3
Group 3 (seven horses)	3	4	1	2
Group 4 (seven horses)	2	3	4	1

Group 1: horses 1-7; group 2: horses 8-14; group 3: horses 15-21; group 4: horses 22-28; NO: novel object; UM: umbrella; HP: horse face poster; PI: pixelated poster.

Recordings taken

Physiological parameters: Heart rate

All horses were habituated to a Polar Equine H7 heart rate monitor, by performing another habituation exercise to the test environment. Habituation to the belt was determined when the behavior of the horse appeared unchanged from without the belt. The week before the tests, a pretest heart rate was measured for 5 min in the test arena when the subject ate but was not exposed to any test stimuli to provide a reference value for each subject. Heart rate recovery (HRR) was determined from the time it took for the subject's heart rate to return to its pretest value + 15% (bpm) and remain at or below this value for five beats, after exposure to the test stimulus (Smith et al., 2016; Ricci-Bonot et al., 2021). If during the test a horse escaped from the test arena in response to the presentation of the stimulus, the session was terminated, and the subject's HRR was imputed as a value of 300 s. Additionally to HRR, heart rate increase (HRI) have been measured by using the difference between the maximum value within 1 min after introduction of the ball or opening of the umbrella and the heart rate average within 3 s before one of these two stimuli. This measurement was used to assess the immediate heart rate response of the subject to the stimulus (Safryghin et al. [2019] and reviewed by von Borell et al. [2007]). The heart rate data were analyzed with the software Kubios HRV 3.0.2. and were checked for artifacts and all recordings with more than 5% heart beat errors were excluded in the HRI analysis (five with the pixelated poster condition and five with the horse face poster condition for the novel object test, and seven with the pixelated poster condition and four with the horse face poster condition for the umbrella test) (Smith et al., 2016).

Behavioral parameters: Reactivity score

Behaviors were recorded continuously by two cameras during testing and were analyzed later by two evaluators separately. Each

Table 1
Description of behaviors contributing to ordinal reactivity scores (adapted from Christensen et al. [2008]).

Score	Reaction	Description
0	None	The horse does not react to the test stimulus and chewing is not interrupted.
1	Head up	The horse raises its head from the food container and chewing may be briefly interrupted, but the horse is not alert (see below) and does not move away from the food container.
2	Alert	The horse stands vigilant with or without elevated neck, with or without tail elevation, head and ears oriented toward test stimulus, chewing is interrupted and the horse may move up to two steps backward or sideways away from the food container.
3	Away	The horse turns, or moves three or more steps backward or sideways away from the food container in response to the test stimulus, typically followed by alertness.
4	Flight	The horse turns/jumps away from the food container in a sudden movement, typically followed by trotting/galloping, alertness and possibly snorting.
5	Escape	The horse leaves the arena.

evaluator rated all videos. The second evaluator was blind to the condition (horse face/pixelated poster).

These cameras (Ordro, 4K, 24 megapixels) were present during the subject's habituation to the test environment.

The behavioral responses of the horses to the novel object (ball) and the opening of the umbrella were evaluated by using ordinal reactivity scores (RSs) adapted from Christensen et al. (2008) and used previously by Ricci-Bonot et al. (2021) (see Table 1). These behaviors are considered to represent an increasing degree of reactivity with the horse presenting an increasing attention toward the stimuli and moving further and further away from the stimuli (with an increase of intensity). After the introduction of the ball into the test area and the opening of the umbrella, the immediate response of the horses to these different stimuli was evaluated by assigning a score (ranked in order of intensity).

Data analysis

Verification of observer agreement on the video scoring (RS)

Inter-reliability between the two behavioral evaluators was assessed using weighted Kappa (R package *irr* and *rel*; R Core Team, 2018).

Analysis of the effect of repeat testing on the behavioral and physiological responses

The first step of the analysis established if there were any within-subjects order effects from the tests on either the subject's behavioral or physiological responses associated with repeat testing. The ordinal data of RS were analyzed with cumulative link mixed models (CLMMs) using the adaptive Gauss-Hermite quadrature (R-package *ordinal*) and the data on HRR and HRI (with cube-root transformation) were analyzed with linear mixed-effect models (LMM) (R-package *lmerTest*). The same factors were used for these two analyses, where the fixed factors were the test type (novel object vs. umbrella) and test order (1, 2, 3, or 4), and the random factor was the identity of the subjects.

Analysis of the effect of the stimulus type on the RS in control conditions

In order to compare the RS obtained during the novel object test (control – pixelated poster) and the umbrella test (control – pixelated poster), a Wilcoxon test was used. This nonparametric test was chosen as data did not follow a normal distribution (Shapiro test).

Influence of visual substitute in novel object and umbrella test

As our focus was the factors affecting the occurrence of social buffering, we considered in our analyses only those horses that showed a behavioral reaction (RS between one and five) when they performed the test with the visual control (pixelated poster), as only in this case was there potential to show a social buffering effect on their responses. To determine if, during stressful situations, the virtual companion reduced the subject's behavioral and/or physiological responses, the RS, and the HRR time when the subject was exposed to the pixelated poster or the horse face poster were compared for each test. As before, CLMM was used for the data on RS and LMM for the data on HRR and HRI, with poster (pixelated vs. horse face) as a fixed factor and the subject's identity as a random factor. The data of HRI have had a cube-root transformation.

The same statistical analyses described above were also run using all horses in order to verify that horses did not find the horse's face poster threatening and therefore possibly increased the stress responses in the subject (i.e., the horse poster led to an increase in reactivity or a slower HRR compared with the pixelated poster [visual control]).

Analysis of the effect of interacting with the poster

In order to determine whether interacting with the horse face poster had an effect on horses' RS, the difference in RS when the pixelated poster and the horse face poster were used was compared between subjects who interacted with the horse face poster (stretching their head or moving toward the horse face poster, and/or trying to make nose-to-nose contact when approaching) and those who did not interact using Mann-Whitney test. This nonparametric test was chosen as data did not follow a normal distribution (Shapiro test).

The data were analyzed with R software (version 3.5.2). The significance of the results was assessed at a threshold of $p < 0.05$ for two-tail predictions.

Results

Verification of observer agreement on the video scoring (RS)

There was high interobserver agreement on the reaction of horses ($k_w = 0.973$ [95% Confidence Interval (CI), 0.948-0.996], $P < 0.0001$).

Habituation performance

The horses needed two trials to be habituated to the test environment. For the first trial, the mean time taken by horses to go to the buckets before eating for 90 s was 70 s (SD = 76.70), and for the second trial, 4.57 s (SD = 1.10). The horses needed only one trial to be habituated to the heart rate equipment. During the experiment, one subject had to be removed because during its second test (novel object with horse face poster) the horse started to show potential signs of increasing distress such as galloping. Only its RS and HRI have been used in the analyses for the novel object test (horse face and pixelated poster).

Analysis of the effect of repeat testing on the behavioral and physiological responses

Repeat testing did not significantly affect the subject's RS nor HRR (RS, CLMM: estimate ± s.e.: −0.17 ± 0.17, $P = 0.332$; HRR, LMM: *t*-value, df: −0.50, 80.061, $P = 0.61$; HRI, LMM: *t*-value, df: −0.024, 67.661, $P = 0.98$).

Analysis of the effect of the stimulus type on RS in control conditions

The median value of RS was one (Quartile 1 [Q1] = 1; Quartile 3 [Q3] = 2) in the novel object test with pixelated poster and significantly higher at four (Quartile 1 [Q1] = 3; Quartile 3 [Q3] = 4) in the umbrella test with pixelated poster (Wilcoxon test: V = 0, n = 27, $P < 0.0001$).

Influence of visual substitute in novel object test

Six horses did not show a behavioral reaction in the novel object test with the pixelated poster. Considering only subjects who showed a reaction with the pixelated poster (22 horses), the median value of RS was one (Quartile 1 [Q1] = 0; Quartile 3 [Q3] = 2) with the horse's face poster and significantly higher at 1.5 (Quartile 1 [Q1] = 1; Quartile 3 [Q3] = 3) with the pixelated poster (CLMM: estimate ± s.e.: 2.15 ± 0.73, $P < 0.004$) (Figure 4a).

For these 22 horses, the mean value of HRR was 40.09 s (SD = 62.53) with the horse's face poster versus 42.91 s (SD = 53.08) with the pixelated poster. Their times to HRR were not significantly different when they were tested with the horse face poster compared to the pixelated poster (LMM: *t*-value, df: 0.164, 21, $P = 0.871$) (Figure 4b).

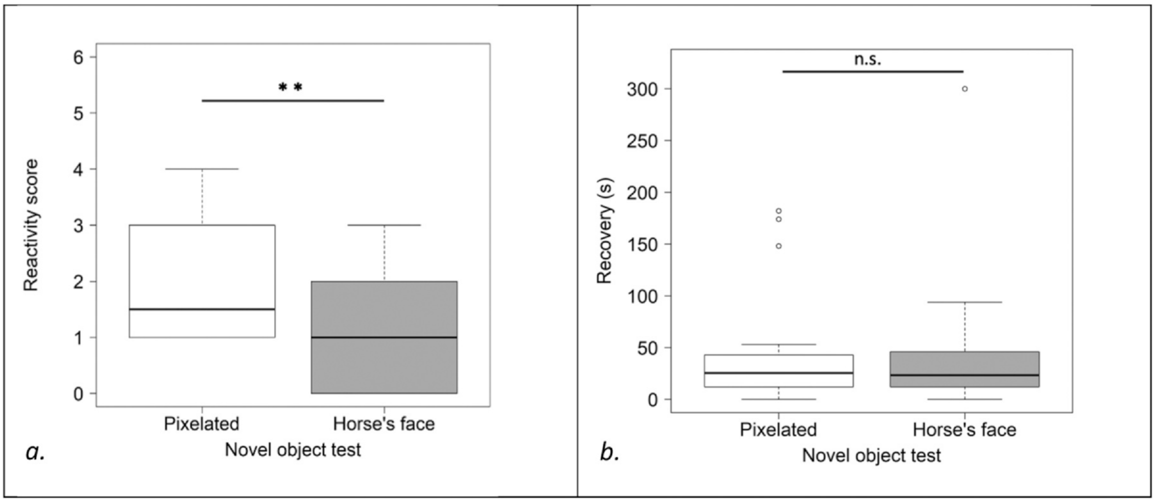

Figure 4. Subject's reactivity score (a) and heart rate recovery (b) in novel object test. (Note: n.s.: $P > 0.05$; $^{**}P < 0.01$; in the "box and whiskers," the bold horizontal line represents the median, the box extends from the first quartile (25th percentile) to the third quartile (75th percentile), the whiskers represent the distribution limits (excluding outliers), and the circle shows the outliers.)

For the HRI, the analyses were run on 16 horses. The mean value of the subject's increased heart rate was 3.09 bpm (SD = 7.16) with the horse face poster whereas it was 7.96 bpm (SD = 12.69) with the pixelated poster. The increase in the subject's heart rate was not significantly different from when it was tested with the horse face poster compared to the pixelated poster (LMM: t-value, df: 1.08, 15, $P = 0.297$).

For all 28 horses in the novel object test, the median RS was 1 with both the horse's face poster (Q1 = 0; Q3 = 1.5) and with the pixelated poster (Q1 = 1; Q3 = 2). There was a significant effect of the horse's face poster compared to pixelated poster on RS in the novel object test (CLMM: estimate ± s.e.: 1.09 ± 0.55, $P = 0.049$). Four horses interacted with the horse's face poster by stretching their head or moving toward the horse face poster, and/or trying to make nose-to-nose contact when approaching. Only half of them had a reduction of their RS compared to when exposed to the pixelated poster.

The mean value of HRR with the horse's face poster was 33.68 s (SD = 56.91) versus 40 s (SD = 49.08) in the novel object test with pixelated poster. There was no significant effect of the horse's face poster compared to pixelated poster on the time to recover (LMM: t-value, df: 0.461, 27, $P = 0.649$).

For the HRI, the analyses were run on 19 horses. The mean value of the subject's increased heart rate was 2.64 bpm (SD = 6.77) with the horse face poster whereas it was 4.75 bpm (SD = 14.06) with the pixelated poster. The increase in the subject's heart rate was not significantly different when it was tested with the horse face poster compared to the pixelated poster (LMM: t-value, df: −0.27, 18, $P = 0.789$).

Influence of visual substitute in umbrella test

All horses showed a behavioral reaction during the umbrella test with the pixelated poster and are therefore included in this analysis. In the umbrella test, the median value of RS in the umbrella test was four with both the horse's face poster (Q1 = 2.5; Q3 = 4) and with the pixelated poster (Q1 = 3; Q3 = 4). Nineteen subjects had no difference in RS regardless of the poster. One subject escaped from the test arena after being exposed to the umbrella with the pixelated poster. There was no significant effect of the horse face poster compared to pixelated poster on RS in the umbrella test (CLMM: estimate ± s.e.: 1.70 ± 0.87, $P = 0.051$) (Figure 5a). Two horses who interacted with the horse's face poster had a lower RS compared to when exposed to the pixelated poster.

The mean value of HRR for these horses was 120.77 s (SD = 109.68) with the horse face poster and 124.48 s (SD = 102.50) with the pixelated poster so the horse's face poster was not associated with quicker HRR (LMM: t-value, df: 0.161, 26, $P = 0.873$) (Figure 5b).

For the HRI, the analyses were run on 18 horses. The mean value of the subject's increased heart rate was 60.50 bpm (SD = 30.28) with the horse face poster whereas it was 63.65 bpm (SD = 37.74) with the pixelated poster. The increase in the subject's heart rate was not significantly different from when it was tested with the horse face poster compared to the pixelated poster (LMM: t-value, df: 0.365, 17, $P = 0.719$).

Analysis of the effect of interacting with the poster

Across the tests, six horses tried to interact with the horse face poster. The difference in RS between subjects who interacted with the poster and subjects who did not was not significant (Mann-Whitney test: W = 184, n1 = 12, n2 = 43, $P = 0.1035$).

Discussion

The picture of an unfamiliar horse face with a relaxed facial expression seemed to have the potential to act as a social buffer depending on the nature of the stressful situation. The horse face picture reduced the subjects' reactivity in comparison to a pixelated control picture in the novel object test. However, we need to be cautious when interpreting these results since there are methodological limitations (discussed below) that might also explain the apparent buffering effect seen here. By contrast, it seems that in the umbrella test, the horse face image did not reduce the subjects' behavioral or physiological response (HRR) compared with the pixelated control picture. A differential effect depending on the type of stressor involved is not surprising and consistent with our previous results (Ricci-Bonot et al., 2021).

The novel object and umbrella stimuli differ in their movement/appearance speed features and their subsequent effect on the size of response: The ball was gradually introduced in the test arena while the umbrella was suddenly opened. This sudden change in the environment can explain the more dramatic reaction (flight response) in our horses during the umbrella test (Górecka-Bruzda et al., 2011).

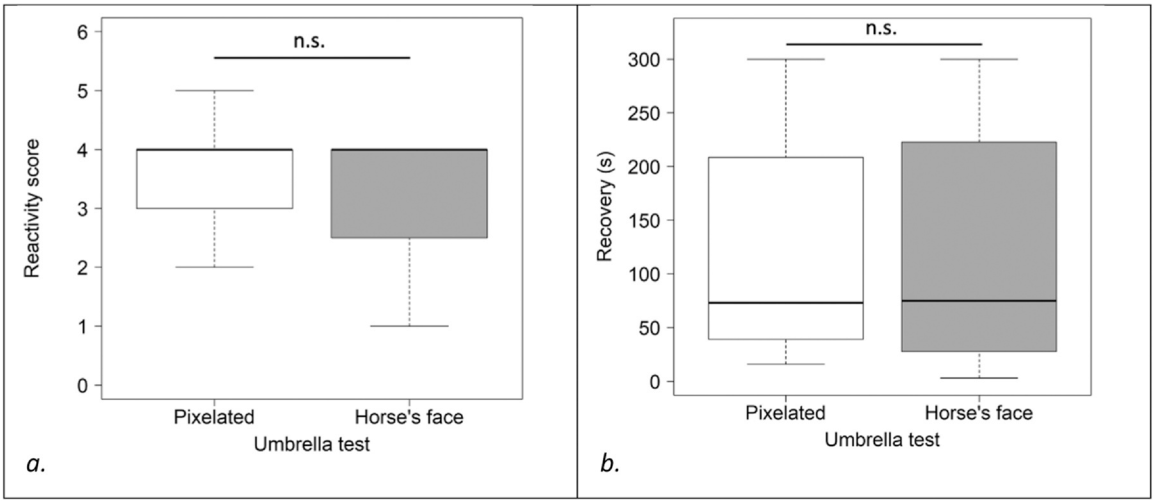

Figure 5. Subject's reactivity score (a) and heart rate recovery (b) in umbrella test. (Note: n.s.: $P > 0.05$).

In horses, the flight response is a natural behavior to avoid a threat (McNaughton and Corr, 2008). The reaction is less dramatic during the novel object test, as the ball/novelty is gradually presented. Therefore, when facing the ball, the subject has time to evaluate the situation to obtain more information about the stimulus and the context in order to produce the most efficient response adapted to the situation (McNaughton and Corr, 2008; Mills et al., 2019). In this case, the horse face picture might act as a point of reference, potentially like a real companion, and thus act as a social buffer (Ricci-Bonot et al., 2021). In contrast, during the opening of the umbrella, it may not be adaptive for the subject to reference other social information before deciding on its response, as there is the potential need for an immediate, rapid response to protect it from harm.

Vandenheede and Bouissou (1994) found that, in sheep, many individuals showed similar behavior towards the image as a real conspecific, that is, they moved toward the extremities of the individual represented in the image, that is, the head and the anogenital region. In the present study, some horses directed behaviors toward the poster after the introduction of the ball or the opening of the umbrella. Six horses tried to interact with the horse face image, either by stretching their head or by moving toward the poster, and in some cases by trying to make nose-to-nose contact when approaching, which in horses is one of the main actions associated with meeting a new conspecific (Feh, 2005). One horse stood alert toward the poster, that is, its neck was elevated, and its head and ears were orientated toward the horse face picture (McDonnell, 2003). It is not surprising that only a few horses interacted with the horse face poster. Indeed, in tests conducted by Mendonça et al. (2019) (such as the opening of an umbrella, presence of an unknown person) where a real horse was present outside the test arena, the authors noted that the horses hardly interacted with each other. However, the absence of this companion resulted in a change in behavior such as a reduction in exploration behavior. Only one horse approached and investigated the pixelated poster, stretching its neck and apparently sniffing it. None of the horses showed fearful reactions towards the pixelated poster during the tests. The pixelated poster, which has the same visual input but without the form of a horse, would not be perceived as a horse and therefore could not serve as a social buffer. It seems therefore that the horse face poster was not perceived simply as a general visual distraction in the environment as potentially suggested by Mills and Riezebos (2005). However, the six horses that tried to interact with the horse face poster were not more likely to have a significant positive social buffering effect compared to others.

During the umbrella test, the horse face poster did not significantly ameliorate the subject's response or recovery. It is possible that, due to the position of the picture, that is, outside the test arena, and the presence of the open umbrella, subjects in the context of the sudden dynamic novelty focused their attention on the stimulus and not the peripheral environment where the poster was. Moreover, after a startle response, the poster was, in effect, located behind the subject in some cases, which may be in the blind spot of the horse (Roberts, 1992; Murphy et al., 2009). These factors might explain why it was more difficult for the poster to act as a social buffer in this context, but it is also possible that the static nature of the horse on the poster meant that there was a lack of meaningful communication with the test subject to help it to cope with the stressful situation. For any social buffering effect to happen during the opening of the umbrella, it may be critical for the companion to show a visible reaction that is noticed by the subject. If the companion reacts, this may communicate to the subject that it has perceived the threat, and a low-level response may convey that the threat is not as great as the subject might otherwise perceive it to be. By contrast, in the novel object test, the poster could have played the role of social buffer because the subject did not go to the other end of the test arena (opposite the stimulus), and/or responses were less intense.

A possible methodological limitation to our study, which we have to acknowledge, is that, although the test order was varied and horses performed only one test per week, it was not possible to provide every possible permutation orders given the number of subjects and tests conditions involved. One consequence was that three of the four test groups received the pixelated poster treatment before the horse face poster treatment. Because horses were exposed twice to each stimulus, it was possible that the habituation could be subject to overlearning effects in the case of the horse face poster. The behavioral responses did, in fact, decrease during the second exposure to the ball for approximately half of the subjects. However, a different pattern of response was noted for HRR with some horses taking a longer time to recover during the second exposure. It is therefore possible that there was a behavioral habituation to the ball but not a physiological habituation. Further research is therefore necessary to confirm the potential phenomenon observed here. Nonetheless, while habituation may have contributed to the buffering effect of the horse face poster, it is unlikely to provide a full explanation of this result. This opinion is in line with a wider consideration of the evidence for social buffering in horses to date. Interestingly, Christensen et al. (2006) in their research on habituation in horses also noted that behavioral habituation came before physiological habituation and the significance of apparent physiological and behavioral habituation responses on future behavioral responses deserves further investigation as it might be of substantial practical importance. For example, just because an individual has behaviorally habituated, it may not have fully habituated to a novel stimulus, and thus might appear to dishabituate more readily at a later date. Future research could also investigate the importance of the companion's behavior during stressful situations on their role as a social buffer. A video is not static and could be used to produce a standardized behavioral response in the virtual companion; it also allows control over the presented sequence, that is, the behavior of the animals in the video as well as the sounds (D'Eath, 1998). Another possible limitation to our study is the use of a single exemplar of a horse's face poster, which prevents the results of this research from being generalized. Therefore, it is not possible to be sure that the lack of observed effects of the horse's face poster is not due to the representation of the one exemplar of the horse's face that we have chosen. For example, we noticed that, in the umbrella test, 19 horses out of 28 showed no effect of the poster, that is, had the same RS with the pixelated poster and the horse's face poster. Among the eight cases where there was an effect on reactivity, for seven of them, there was a reduction in RS with the horse's face poster. Perhaps the use of a second poster would have had an effect on the 19 subjects who did not present a changing of behavior. Consequently, future research need to use at least two different horse's face poster in order to be able to generalize their results. Moreover, in order to improve the procedure, it might have been better to ask experts to comment on the fourth horse's face picture before use, but we all judged the horse to look relaxed according to the criteria presented by the experts.

This research showed that the social buffering function of a companion might in some circumstances be at least partially replaced by a visual substitute. Any effect of a poster of a relaxed horse's face seems to be on reducing the behavioral response when the subject faces a gradual change in novelty, rather than a sudden change in stimuli. Further work should explore the practical value of these observations.

Authorship statement

CRB, TR, CN, and DM conceived and designed the experiments, and wrote the paper. CRB performed the experiments, analyzed, and interpreted the data with the support of the other authors. All authors gave final approval for publication and agree to be held accountable for the work performed.

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Conflict of Interest

The authors declare no competing interests.

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