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Does training style affect the human-horse relationship? Asking the horse in a separation–reunion experiment with the owner and a stranger

Paulina Lundberg [a], Elke Hartmann [b], Lina S.V. Roth [a,*]

[a] IFM Biology, Linköping University, 581 83, Linköping, Sweden
[b] Department of Animal Environment and Health, Section of Ethology and Animal Welfare, The Swedish University of Agricultural Sciences, 75007, Uppsala, Sweden

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ABSTRACT

Humans have shared a long history with horses and today we mainly consider horses as companions for sports and leisure activities. Previously, the human perspective of the human-horse relationship has been investigated but there has been little focus on the horse's perspective. This study aimed to reveal whether horses show attachment-related behaviour towards the owner compared to a stranger in a modified Strange Situation Procedure (SSP) consisting of a walking phase, a standing still phase, separation from the owner/stranger and lastly a reuinon. We tested 26 privately owned horses in an indoor experimental area of 20 × 14 m. In addition to testing, the owners were asked questions about their training methods. Based on these questionnaire results, owners were divided into groups depending on whether they mainly used negative reinforcement, positive reinforcement or a combination of both methods during training. They also completed a horse personality questionnaire. The results showed that the horses spent more time in door proximity when separated from the owner and the stranger (owner: $Z = -3.46$, $P = 0.001$; stranger: $Z = 3.40$, $P = 0.001$) compared to the reunion phase, and they sought human proximity during reunion. The horses' heart rates were higher during the separation compared to the reunion with both the owner ($Z = -3.44$, $P = 0.001$) and the stranger ($Z = -2.40$, $P = 0.016$). These results are examples of attachment-related features and suggest that horses consider both the owner and the stranger as a safe haven. However, the results are not clear as to whether or not horses perceive their owners as a secure base since their exploratory behaviour during owner reunion was similar to that during stranger reunion. Interestingly, horses trained with positive reinforcement spent most time in door proximity during separation from the stranger ($\chi^2(2) = 6.18$, $P = 0.045$) and similarly there was a tendency also during owner separation ($\chi^2(2) = 5.20$, $P = 0.074$). The same group of horses also spent more time in stranger proximity ($\chi^2(2) = 6.16$, $P = 0.046$) and in physical contact with stranger ($\chi^2(2) = 8.62$, $P = 0.013$) than the other two training style groups during reunion. When correlating scores from the horse personality questionnaire with behaviours during owner reunion, we found few significant associations, but the trait *Inquisitive* correlated with both proximity to owner and ears forward ($rs = 0.41$, $P = 0.035$ and $rs = 0.49$, $P = 0.011$, respectively), and ears forward also correlated with the trait *Excitability* ($rs = 0.39$, $P = 0.047$) and *Dominance* ($rs = 0.46$, $P = 0.019$). Hence, this study revealed attachment-related behaviours of horses towards humans even though the results cannot resolve whether these fulfil all criteria for an attachment-bond.

1. Introduction

Humans and horses share a long history during which the roles of the horse as a food source, for transportation and work have progressively made way for those of sports and leisure purposes, for companionship or therapeutic reasons. Many riders and horse owners would consider their horse as part of the family and would relate to it as they would to a child or to another family member. They may also refer to the relationship as one marked by mutual trust, respect and affection (Mills and Nicholas, 2005). Yet, while the scientific focus has been on the formation of emotional bonds from humans to horses (DeAraugo et al., 2014), the horses' perspective on the relationship has rarely been investigated. Thus, the question remains whether horses show attachment-related behaviours towards humans that fulfil the four features of attachment, namely proximity seeking (i.e. preferring to be near the attachment figure in times of stress), safe haven (i.e. relief from stress due to the

* Corresponding author.
E-mail address: lina.roth@liu.se (L.S.V. Roth).

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comfort and support provided from the attachment figure), secure base (i.e. increased exploration due to feeling safe), and separation distress (i.e. feeling distressed in the absence of the attachment figure). This topic has important implications for the behaviour and welfare of both dyad members and for equine performance during training. For example, clarity in this domain will assist in demystifying the concepts of trust and respect and moderate inappropriate human expectations in training contexts. Alternatively, trust in humans based on an attachment bond could help to reduce a horse's arousal in a frightening situation (McLean et al., 2013).

The existence of attachment bonds of animals towards humans has mainly been explored in companion dogs and recently also cats (Payne et al., 2015; Rehn et al., 2014; Vitale et al., 2019), using established methods derived from human psychology to investigate the attachment between toddlers and their parents (Ainsworth and Bell, 1970). The so-called Ainsworth Strange Situation Procedure (ASSP) test includes exposing the child to challenging events such as separation and reunion from the parent and meeting an unfamiliar person in a novel environment in order to study the child's proximity seeking to an attachment figure in times of distress (Bowlby, 1958, 1982). According to Ainsworth and Bell (1970), attachment can be defined as "an affectional tie that one person or animal forms between himself and another specific one - a tie that binds them together in space and endures over time". Attachment behaviour is described by, e.g. Bowlby (1982) as:"any form of behaviour that results in a person attaining or maintaining proximity to some other clearly identified individual who is conceived as better able to cope with the world".

Since most dogs live together with their owner in the same household, long-term stable relationships can develop, sharing some features of the child-parent bond (Topál et al., 1998, 2005). By using a modified version of the ASSP, referred to as the Strange Situation Procedure (SSP), the quality of the relationship from the animal's perspective can be measured. During the SSP, it has been shown that dog-human dyads share the four fundamental features typical of an attachment bond (Payne et al., 2015; Rehn et al., 2013; Gácsi et al., 2013): 1) the dog shows separation-related distress in the absence of the attachment figure, 2) the dog seeks proximity to the attachment figure in order to cope with stress, 3) the attachment figure buffers the stress of the dog, and 4) the presence of the attachment figure activates exploration of the environment.

However, there are species-specific differences between dogs and horses and how they are kept that we need to consider to understand possible behavioural differences in these human-animal relationships. Dogs usually share their everyday life together with their owner, often living under the same roof whereas horses live with conspecifics and mainly interact with humans for a limited time of the day. Therefore, we would expect differences in the relationship towards humans between these two species but also similarities as both dogs and horses are social animals. Certainly, the development of a bond between a horse and its owner is a desired goal of many training philosophies. Søndergaard and Jago (2010) found that flight distances were shorter of foals handled early in life in the presence of their dams than those of unhandled foals. The attenuated stress levels in the foals may reflect positive associations made with humans, or the mare could have acted as a safe haven instead of the human. Also, results from Ijichi et al. (2018) could not give evidence of a secure base effect in horses when they were handled by their owners compared to a stranger during a novel handling test. Hence, the human-horse bond needs to be more thoroughly evaluated.

As mentioned earlier, the type of handling and training methods used can have a profound effect on any animal-human relationship. In dogs, a reward-based training method, i.e. using mainly positive reinforcement (adding something pleasant such as food or pleasant tactile interaction to reward a desired behaviour, PosRe) instead of positive punishment (adding an unpleasant stimulus to stop an undesired behaviour) and negative reinforcement (NegRe, application of an unpleasant stimulus that is removed upon desired response) has been found to improve dog-human attachment (Vieira de Castro et al., 2019). Compared to most dog training, using PosRe has only recently been introduced in horse training (McLean and Christensen, 2017) which has traditionally been dominated by NegRe. Sankey et al. (2010a; 2010b; 2010c) studied the effect of PosRe training on the horse-human relationship. They found that horses spent more time close to the trainer than to an unfamiliar handler and approached the trainer faster when receiving a food reward than horses trained without food or trained with NegRe (Sankey et al., 2010a). Notably, these effects could be explained by the use of PosRe that may have promoted bonding as suggested by the authors. Alternatively, approach behaviour may have mirrored the horses' expectation and motivation to receive food. The authors also suggest that tactile contact (vigorously grooming the withers three times) was insufficient for bonding to occur (Sankey et al., 2010c), measured by the latency to approach a human. Indeed, scratching the withers vigorously may not be perceived by horses as sufficiently positive or rewarding to facilitate human-animal bonding. However, tactile contact seems important in affiliative horse-horse interactions and empirical evidence suggests that this may also apply to human-horse interactions, i.e. prolonged and gentle grooming at the horses' preferred sites can decrease heart rates and induce more relaxed type of behaviours (Feh and de Mazières, 1993; McBride et al., 2004; Wolter et al., 2018).

The aim of this study was to investigate whether horses show behavioural and physiological (heart rate) responses that reflect the four features typical for the attachment bond, i.e. separation-related distress, proximity seeking upon reunion, stress buffering, and increased exploration, in a simplified SSP with their owner and a stranger. Furthermore, owner-horse dyads using different training methods, i.e. NegRe, PosRe or combined reinforcement (CRe) were recruited to reveal possible effects of different training methods on the horses' human-directed behaviours. In dogs, their personality has been shown to influence features of their attachment towards the owner (Solomon et al., 2019). Therefore, we also wanted to assess the horses' personality through a questionnaire to reveal whether certain personality traits correlate to the human-directed behaviours.

2. Methods

Experimental procedures conformed to the guidelines for the ethical treatment of animals in applied animal behaviour research (http://www.applied-ethology.org/ethical_guidelines.html). No special ethical permit was needed according to Swedish regulations.

2.1. Horses, owners and experimental areas

Twenty-six privately owned horses and their owners (all females) were included in this study, and one owner participated with two horses. The horses consisted of 14 mares and 12 geldings of different breeds and age, ranging from 4 to 28 years (Mean 13.0 ± 1.2 years). The study was performed between September-November 2018 in privately owned stables and indoor riding arenas in the region of Linköping in the southeast of Sweden.

Participants were recruited through social media and personal contacts, and all owners had owned their horse for at least one year. They were informed about the study's aims and procedures and gave their written consent to participate.

2.2. Owners' training style

In order to investigate whether the owners' training styles influenced the behaviours of the horse during the modified SSP, the owners were asked to complete a questionnaire regarding their training style (Table 1). Answers were used to classify owners into three different groups: mainly using negative reinforcement (NegRe), mainly using positive reinforcement (PosRe) or using a combination of both negative and positive reinforcement (CRe). The questionnaire consisted of 22

Table 1
Questions included in the training style questionnaire to be able to classify owners into three different groups based on whether they mainly use negative reinforcement (NegRe), positive reinforcement (PosRe) or a combination of both negative and positive reinforcement (CRe).

Questions
I use pressure and release when I want to teach my horse something new.*
As soon as my horse performs a correct behaviour I release the pressure.*
When I want my horse to move forward I use my driving aids.*
If my horse doesn't respond to my aids I emphasise them by intensifying the pressure.*
I sometimes gently touch my horse with the whip to clarify what I want it to do.*
If my horse refuses to move forward I add the whip.*
When my horse is afraid of something I help it by being consequent with my driving aids *
If my horse performs an unwanted behaviour I correct it *
I use treats when I want to teach my horse something new.#
I always give my horse a treat when it performs a correct behaviour.#
When I want to teach my horse something new I wait until the horse offers the behaviour by itself.#
I use treats if I want my horse to overcome its fear of something.#
I play with my horse in order to lure out new behaviours for which I can give treats.#
If my horse refuses to move forward I lure with a treat.#
If my horse performs an unwanted behaviour I ignore it.#
I use treats in the company of my horse.#

* Related to negative reinforcement or positive punishment.
\# Related to positive reinforcement or negative punishment.

Likert-scale questions (1–5), of which eight questions were related to the use of NegRe in training and eight questions were related to using PosRe (Table 1). Six additional questions were incorporated into the questionnaire only with the purpose to balance the questions and to keep the owner with a positive attitude throughout the questionnaire (e.g. "I give treats in a bucket/feeder" and "My horse comes when I call").

The questionnaire was piloted with eight female horse owners not participating in this study. Results showed a highly significant correlation between the test-retest reliability ($r < 0.80$, $P < 0.001$).

The training style questionnaire was further validated with a training test that was performed by the owners and their horses on the experimental day. To avoid any pre-training, the owners only received information about the training test on the experimental day. In addition, to avoid any bias, the owner was only told that this test aimed to study the behaviour of the horse in different situations and not that it was used for validating the training style questionnaire. The training test consisted of five tasks, always in the same order, where the owner was asked to make her horse: 1) step backwards, 2) step forward, 3) step sideways to the left, 4) step sideways to the right, and 5) touch a novel object (a flyswatter). The owner was instructed to perform the tasks as she normally would do in her daily care of the horse, but the start position of the owner was always face-to-face with the horse. If the owner did not succeed with a task, the experimenter interrupted after one minute and asked the owner to continue with the next task. Horses wore a regular halter and the training test was performed in the indoor riding arena. The test was video recorded (Canon Legria) for later analysis of the owner's behaviour toward the horse.

2.3. Horses' personality questionnaire

In order to test whether the personality of the horse affected its behaviour during the modified SSP, all owners were asked to complete the horse personality questionnaire (HPQ) developed by Lloyd et al. (2007). The HPQ consisted of 25 fully defined adjectives where the owner was asked to score their horse from 1 (not expressed) to 7 (fully expressed) for each adjective (i.e. reliable, subordinate, equable, eccentric, effective, stubborn, aggressive, irritable, suspicious, insecure, tense, apprehensive, fearful, active, slow, excitable, intelligent, understanding, motherly, protective, sociable, playful, popular, curious, opportunistic).

2.4. Experimental arena and test preparations

The experiment was performed in 16 different indoor riding arenas measuring 20 × 40 m or 20 × 60 m. Twenty-three of the 26 horses were familiar with the arena, and the remaining three horses had experience of visiting new arenas 1–5 times per year. Nine out of 26 horses were transported by trailer to the indoor riding arena. A 20 × 14 m experimental arena was fenced off from the indoor arena using jumping stands and white/red warning tape. Two full-HD camcorders (Canon Legria) were positioned outside the experimental arena, covering each half of it.

The owners were informed beforehand about the experimental procedure and they also received additional illustrative instructions on the experimental day. In addition, the owner was allowed to use a step-by-step guide printed on paper during the actual testing. The owner was asked to wear a helmet and a stopwatch (Casio HS-80TW) in order to keep track of the time for the different test phases.

During the test day, the horse was only allowed to feed on roughage. In preparation for testing, the horse was tied up in a halter in the stable aisle and the owner equipped the horse with a Polar Equine belt with an attached H7 sensor (Polar®). Water (25−35 °C) was applied as an electro facilitator on the left side of the horse, from the withers down to the girth area where the Equine Polar belt electrodes were positioned. The heart rate of the horses was monitored by a Polar® sports watch (V800) which was fastened on the halter. Before the owner and the horse entered the indoor arena, the sports watch and the stopwatch were synchronised and started simultaneously by the female experimenter.

2.5. Experimental procedure

The main aim of the experiment was to compare the behaviour of the horse during a modified SSP with both the owner and a stranger. All horses were tested with the owner first followed by the same procedure with the stranger (the female experimenter). None of the horses had been in the riding arena on the day prior to testing. Thus, the fenced off experimental arena was regarded as novel for the horses.

The experiment consisted of four phases (for description see Table 2): 1) walking, 2) standing still, 3) separation, and 4) reunion. The owners were asked to behave passively and relaxed towards the horse during all phases, and they were not allowed to talk. When returning to their horse during the reunion, the owner was instructed to walk to either the left or the right corner when entering the experimental arena, depending on the position of the horse. For example, if the horse was close to the left corner, the owner walked to the right corner and if it stood at the door

Table 2
Schematic overview of the experimental procedure. All four test phases were applied in the same order, first with the owner and then with the stranger.

Phase	Duration	Procedure
Walking	180 s	Owner/Stranger chose direction and walked around the outer lap of the experimental arena with the horse on a loose hanging lead rope. If the horse stopped, the owner/stranger was instructed to gently negatively reinforce the horse to walk again by pulling the lead rope.
Standing still	60 s	Owner/Stranger positioned herself on the X-mark (an X drawn in the ground substrate in the middle of the experimental arena), facing the door, holding the horse on a loose lead rope. The horse was allowed to move around as far as the lead rope would allow it. This phase started as soon as the owner was positioned on the mark.
Separation	120 s	After one minute, the owner/stranger detached the lead rope and left the experimental arena to get out of sight of the horse. The horse was left alone and free to move in the experimental arena.
Reunion	120 s	Owner/Stranger returned and walked to the corner to either the left or right of the door. While in the corner, the owner was instructed to stand passively but was allowed to look at the horse. The horse was free to move in the arena.

upon entering, the owner was instructed to choose the corner most easily reached, without engaging in face-to-face contact with the horse.

After this first part of the experimental procedure with the owner, the Equine Polar belt was removed by the owner and the horse was allowed to have a break for approximately 15 min in its home box or transport. After this break, the horse was again equipped with the Equine Polar belt by the owner to minimise contact with the female experimenter prior to testing. The same test procedure was repeated in the experimental arena, but this time with the female experimenter acting as a stranger.

2.6. Behavioural recordings and heart rate

The behaviour of the horses was analysed from the video recordings in the software Noldus Observer XT (version 13) according to the ethogram in Table 3. The horses' behaviours and positions were recorded as duration (in s) except ear flickering and vocalisations (frequency or total number). Each phase (walking, standing still, separation, reunion) was analysed separately (Fig. 1). The analysis of the walking phase (120 s) began when the owner/stranger started to walk with the horse in the experimental arena after having closed the door of the indoor arena. The start of the standing still phase (45 s) was calculated backwards from when the owner/stranger closed the door and left the indoor arena in order to capture whether the horse would follow. The separation phase (120 s) started one second after the owner/stranger had closed the opaque door behind her. The reunion phase (90 s) started directly when the owner/stranger returned and stepped inside the experimental arena (Fig. 1).

The heart rate was recorded as beats per second and the mean heart rate (beats per min) was calculated for each horse and phase (walking, standing still, separation, reunion).

Table 3
Ethogram of behaviours and positions (duration in s) of the horse during all four test phases: walking, standing still, separation and reunion.

	Functional term	Description
Behaviour		
	Walking	Moving in any forward direction in a four-beat gait with a diagonal sequential movement.
	Trotting	Moving in any forward direction in a two-beat gait where the legs are synchronised diagonally.
	Canter	Moving in any forward direction in a three-beat asymmetrical gait.
	Physical contact	Any part of the horse's head is touching the owner/stranger.
	Human attention	Horse's head is directed towards owner/stranger with both ears directed forward for at least 1 s.
	Ear forward	Both ears of the horse are positioned forward for at least 1 s or more.
	Ear flicker*	Either of the horse's ears changes from one position to another.
	Head high	Horse's poll is positioned above the withers.
	Head low	Horse's poll is positioned below the withers, but the muzzle is not in close proximity nor in contact with ground.
	Exploring	Horse's muzzle is positioned close to the ground, can also be in contact with the ground.
	Pawing	Front leg is lifted, then extended quickly in a forward direction, followed by a movement backward dragging the toe against the ground in a digging motion.
	Head roll #	The horse tosses its head in a circular upward movement. Usually starting at chest or low and then tossing the head upwards as the head twists around the poll.
	Excretion #	The horse defecates or urinates.
Vocalisation		
	Neighing	The horse generates a medium pitched sound.
	Snorting #	The horse generates a vibrating low pulsing exhalation sound.
	Snoring #	The horse generates a short raspy inhalation sound.
Horse's position		
	Door proximity	Within 1.5 m of the door.
	Human proximity	Within one horse's body length of the owner/stranger.

* Recorded as frequency.
\# Recorded as total number.

2.7. Questionnaires

The training questionnaire was correlated with the summed score from the training style test where the owner was given, for each task, one point for using NegRe, three points for using PosRe and two points for CRe.

The horse personality questionnaire generated for each horse one score for each of the 25 adjectives. These scores were thereafter multiplied with the original Principal Component Analysis loadings according to Lloyd et al. (2007) to achieve values on six different personality traits: *Dominance* (high negative loadings for reliability, subordinate and equable, and high positive loadings for irritable, aggressive, stubborn, effective and eccentric), *Anxiousness* (high positive loadings for fearful, apprehensive, tense, insecure and suspicious), *Excitability* (high negative loading for slow and high positive loading for active, intelligent and excitable), *Protection* (high loadings for protective, motherly and understanding), *Sociability* (high loadings for popular, playful and sociable) and *Inquisitiveness* (high loadings for opportunistic and curious).

2.8. Statistical analyses

All statistical analyses were performed with the software SPSS (version 25 and 26). Non-parametric tests were performed for all behavioural variables and heart rate measurements since they differed from normal distribution (Shapiro-Wilk, $P < 0.05$). For comparisons between the owner and stranger test, and comparisons between test phases, Wilcoxon signed rank tests were used, and for group comparisons (training style group) Kruskal-Wallis H tests were performed. When significant differences were found between groups with Kruskal Wallis H tests, pairwise comparisons were performed adjusted by the Bonferroni correction for multiple testing and only significant results from these tests are reported.

The frequency data for ear flickering was normally distributed in all phases (Shapiro-Wilk, $P > 0.1$) and was therefore analysed with Paired samples T-test for related comparisons (comparisons between owner and stranger test, and comparisons between test phases). Also, the scores for all personality traits were normally distributed and tested with Anova test, except for the trait *Protection* (Shapiro-Wilk, $P = 0.045$) where Kruskal–Wallis H test was used. Behavioural correlations were performed with Spearman's correlations due to the non-parametric distribution of the data. Pearson's correlation was used for the test-retest reliability and validation of the training style questionnaire since the training style scores were normally distributed (Shapiro-Wilk, $P = 0.14$). Mean, SE, P-values and test statistics are reported for all results.

3. Results

3.1. Behaviour together with owner vs stranger

During the walking phase, the horses kept their heads high for a significantly longer duration with their owner compared with the stranger ($Z = -2.65$, $P = 0.008$), and explored more together with their owner than with the stranger ($Z = -2.19$, $P = 0.029$; Fig. 2A). In addition, the horses showed a significantly higher frequency of ear flickering with their owner (Mean 42.2 % ±3.7) than with the stranger (Mean 31.5 ± 3.1 %; $t = 2.57$, $P = 0.016$). During the standing still phase, there was a tendency for the horses to explore more together with

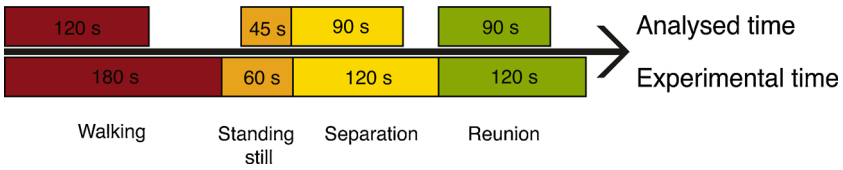

Fig. 1. Schematic view of the four experimental phases and analysed durations.

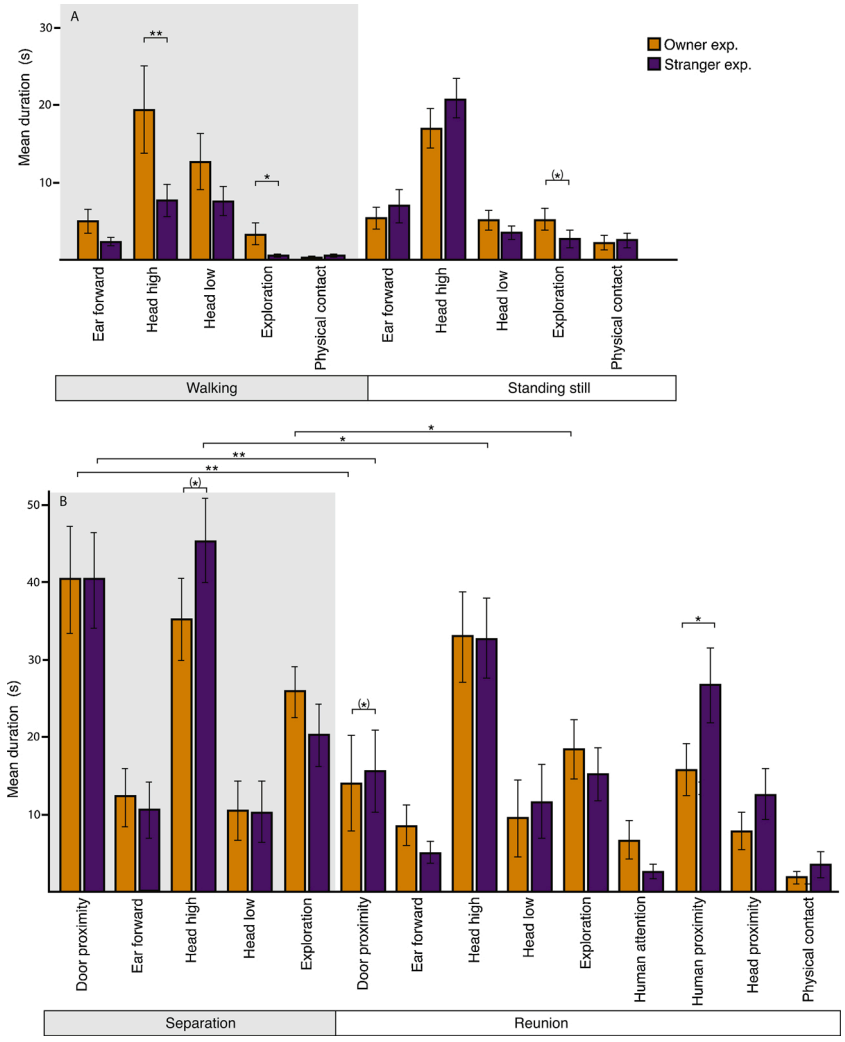

Fig. 2. Mean duration (s) ±SE of the horses' behaviour during the A) walking (shaded area) and standing still phase, and B) separation (shaded area) and reunion phase with owner (orange bars, always to the left) and stranger (purple bars, always to the right). Significant differences are indicated by (*)$p < 0.1$, *$p < 0.05$, **$p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

the owner than with the stranger ($Z = -1.76$, $P = 0.079$; Fig. 2A).

During the separation phase, we found a weak trend for the horses to keep their head high for a longer duration after the stranger left compared to when they were left by the owner ($Z = -1.69$, $P = 0.091$; Fig. 2B). During the reunion phase, the horses spent significantly more time in stranger proximity compared to the owner ($Z = -2.27$, $P = 0.023$; Fig. 2B) and there was also a tendency that the horses spent more time in door proximity during stranger reunion compared to owner reunion ($Z = -1.82$, $P = 0.069$; Fig. 2B).

The duration of walking (Fig. 3) and trotting was similar ($P > 0.1$) comparing the different phases with the owner to those with the stranger. Trotting was recorded for one horse during the walking phase with the stranger (2.64 s), three horses during the owner separation (Mean 2.78 ± 1.89 s) and for four horses during the stranger separation (Mean 4.72 ± 2.75 s). During the owner reunion, three horses trotted (Mean 0.88 ± 0.49 s), while two horses trotted during the stranger reunion (Mean 0.48 ± 0.36 s). Canter was only registered for one horse (0.69 s) during the separation from the stranger.

Head rolls occurred 11 times during the whole owner experiment and 21 during the whole stranger experiment and did not differ between owner and stranger phases ($P > 0.1$). Similarily, few horses pawed, i.e. in total eight horses performed short durations of pawing behaviour during the owner experiment and only one horse pawed during the stranger experiment . Excretion did not occur.

3.2. *Comparing separation and reunion behaviour*

The horses spent significantly more time in door proximity during

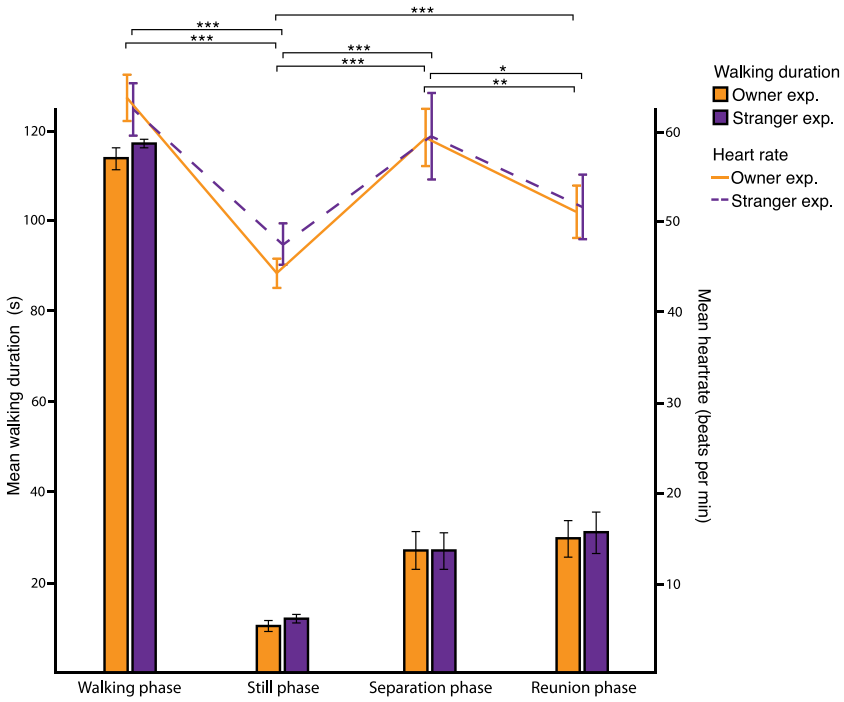

Fig. 3. Mean walking duration (s) ±SE and mean heart rate (beats per min) ±SE during all phases with both owner (orange) and stranger (purple). Significant heart rate differences are indicated by *p < 0.05 **p < 0.01, ***p < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

the separation phase compared to the reunion phase whether tested with the owner (Z = −3.46, P = 0.001) or the stranger (Z = 3.40, P = 0.001; Fig. 2B). They also held their heads high during a longer period of time in the separation phase compared to the reunion when tested with the stranger (Z = −2.50, P = 0.012) but not with the owner (Z = −0.72, P = 0.47; Fig. 2B). The horses showed more exploratory behaviour during the separation phase compared to the reunion phase when tested with the owner (Z = −2.01, P = 0.045) but not with the stranger (Z = −0.93, P = 0.35; Fig. 2B). There was a strong tendency for the horses to flick their ears more during the reunion phase than during separation from the owner (t = −2.03, P = 0.053, separation Mean = 27.09 ± 3.20 %, reunion Mean = 32.51 ± 3.23 %) but not with the stranger (t = −1.52, P = 0.14, separation Mean = 30.64 ± 3.68 %, reunion Mean = 34.92 ± 3.02 %). No significant difference was found for the duration of walking between separation and reunion phase (Fig. 3), regardless of being tested with the owner (Z = −0.78, P = 0.44) or stranger (Z = −0.90, P = 0.37).

3.3. Vocalisations

Few horses vocalised while walking, and since the distribution of the number of vocalisations was few and distributed very uneven during this phase, it was not statistically analysed. Seven horses snorted once each while walking with the owner and five horses snorted in total seven times while walking with the stranger. Fiftyfour snores from only three horses were recorded while walking with the owner and nine snores from two horses during the walking phase with the stranger. Only one horse neighed during both the owner and stranger walking phase. No vocalisation occurred during the standing still phase.

During the separation from the owner, seven horses snored in total 30 times and during separation from the stranger eight horses snored, which was significantly more than during the standing still phase (owner: Z = −2.41, P = 0.016; stranger: Z = −2.14, P = 0.032). Three horses snorted in total five times when separated from the owner, but none during the stranger separation, which is similar to the standing still phase (owner: Z = −1.63, P = 0.10; stranger: Z = 0.00, P = 1.00). Three horses neighed during both the owner and stranger separation phase.

During owner reunion, two horses snorted once each and four horses snored in total 20 times, which is similar to the separation phase (snort: Z = −0.82, P = 0.41; snore: Z = −1.15, P = 0.25). During stranger reunion, four horses snorted once each and two horses snored in total three times, which was significantly different from the separation phase (snorts: Z = −2.00, P = 0.046; snore: Z = −2.39, P = 0.017). One and two horses neighed during the owner and stranger reunion, respectively.

3.4. Heart rate

There was no difference in the horses' heart rates comparing the different phases with the owner to the corresponding phases with the stranger (walking: Z = −0.70, P = 0.48, standing still: Z = −0.91, P = 0.37, separation: Z = −0.62, P = 0.54, and reunion: Z = −0.57,P = 0.57; Fig. 3).

There was a significant decrease in the horses' heart rates between the first phase walking and the second phase standing still with both owner and stranger (owner: Z = −4.38, P < 0.001, stranger: Z = −3.37, P < 0.001; Fig. 3). Between the phase standing still and the third phase separation, there was a significant increase in heart rate (owner: Z = −4.23, P < 0.001, stranger: Z = −3.69, P < 0.001), and then the heart rate of the horses decreased again between the separation phase and the reunion phase (owner: Z = −3.44, P = 0.001; stranger: Z = −2.40, P = 0.016; Fig. 3).

Comparing the first phase walking and the third phase separation revealed that the heart rate of the horses tended to be lower during the separation compared to the walking phase when tested with the owner (Z = −1.90, P = 0.058) but there was no significant difference between the two phases when tested with the stranger (Fig. 3; Z = −1.30, P = 0.19; Fig. 3). When comparing the second phase standing still and the fourth phase reunion, there was a significant increase of the horses' heart rates in the reunion phase when tested with the owner (Z = −3.64, P < 0.001) but not with the stranger (Z = −1.42, P = 0.16; Fig. 3).

3.5. Training style questionnaire

The owners' answers from the training style questionnaire (Table 1) were significantly correlated with the scores from the owners' behaviour during the validation test (N = 26, rs = 0.73, p < 0.001). Thus, owners could be reliably divided into three training style groups according to the questionnaire scores: mainly using NegRe (N = 4, score 0–12), mainly PosRe (N = 9, score 25–36) and the group using CRe (N = 13, score 13–24).

3.6. Effect of training style on behaviour and heart rate

During separation, when the horse was left alone, there was a significant difference in door proximity between training groups when tested with the stranger ($\chi^2(2) = 6.18$, P = 0.045). Additional pairwise comparisons revealed a tendency (P = 0.081) that the CRe-group spent less time in door proximity than the PosRe group during the separation phase (Fig. 4A). There was also a tendency that the training groups differed in door proximity during the separation phase when tested with the owner ($\chi^2(2) = 5.20$, P = 0.074; Fig. 4A). During reunion, there was no difference in door proximity between training groups regardless of being tested with the owner ($\chi^2(2) = 1.74$, P = 0.42) or the stranger ($\chi^2(2) = 1.32$, P = 0.52; Fig. 4B).

Focusing on the human-related behaviours in the reunion, there were significant differences between training style groups for head proximity to the stranger ($\chi^2(2) = 6.16$, P = 0.046; Fig. 4C) and physical contact with the stranger ($\chi^2(2) = 8.62$, P = 0.013; Fig. 4D). Additional pairwise comparisons revealed that the PosRe group spent more time compared to the NegRe group in both head proximity (P = 0.050) and physical contact (P = 0.020). Analysing only the data from horses in the PosRe group (N = 9) revealed that they had more physical contact with the stranger than with the owner during the reunion (Z = −2.38, P = 0.017; Fig. 4D) and, similarly, there was a tendency for the PosRe group to spend more time in head proximity with stranger compared to head proximity with owner (Z = −1.72, P = 0.086; Fig. 4C). Testing only the horses in the NegRe group revealed no significant differences between the owner and stranger test for neither physical contact (Z = −1.10, P = 0.27) nor proximity (Z = −1.34, P = 0.18) during reunion.

Comparing the mean heart rates between the training style groups in all test phases with both owner and stranger revealed no significant differences (P > 0.1).

3.7. Personality

Personality scores revealed few associations with the observed behaviour of the horse. Focusing on the owner reunion, proximity to owner and ears forward correlated positively with the trait *Inquisitive* (rs = 0.41, P = 0.035 and rs = 0.49, P = 0.011, respectively). Ears forward also correlated with the trait *Excitability* (rs = 0.39, P = 0.047) and the trait *Dominance* (rs = 0.46, P = 0.019). During the stranger reunion, ear flickering correlated with *Inquisitive* (rs = 0.53, P = 0.005), *Sociability* (rs = 0.40, P = 0.045), and *Excitability* (rs = 0.51, P = 0.008).

There were significant differences in the *Dominance* trait among training style groups (F(2, 25) = 4.44, P = 0.023) where the NegRe

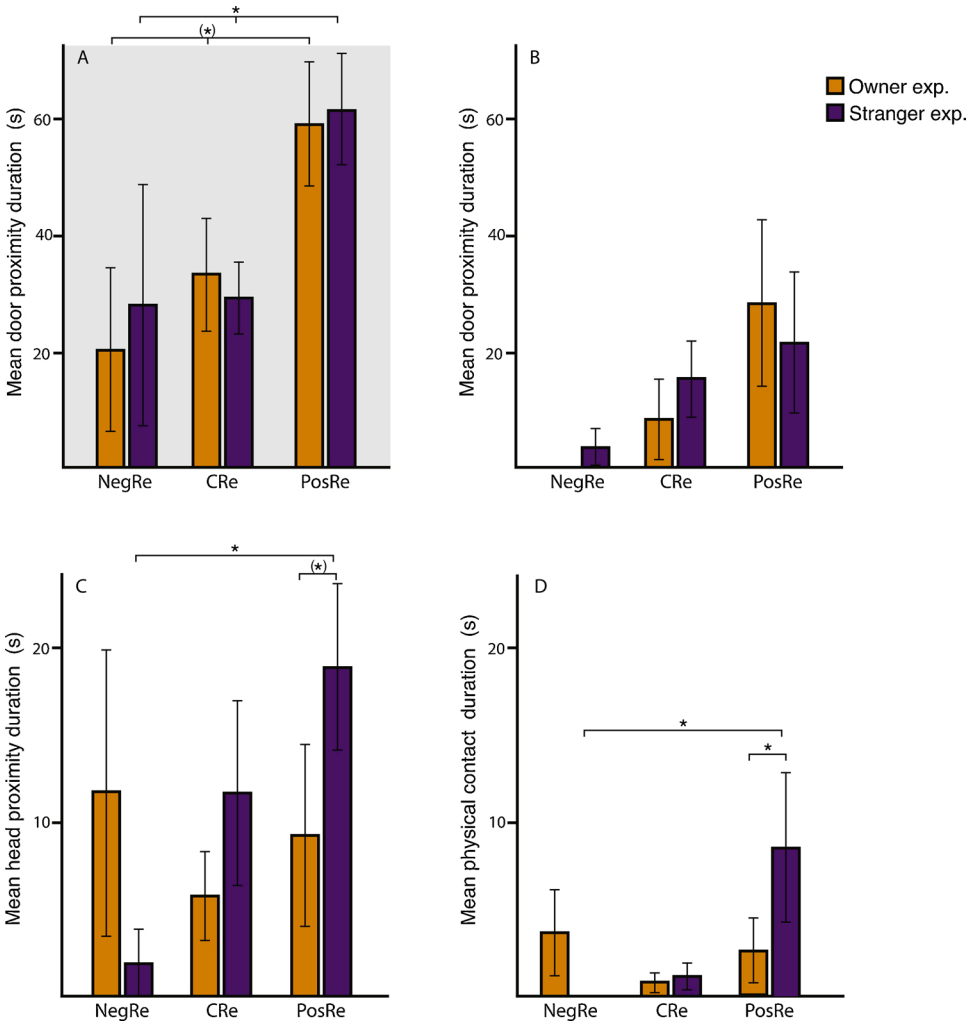

Fig. 4. Mean duration (s) ±SE of door proximity during A) separation (shaded area) and B) reunion, and C) head proximity and D) physical contact during reunion with both owner (orange bars, always to the left if recorded) and stranger (purple bars, always to the right if recorded) for horses in Negative reinforcement group (NegRe, N = 4), Combined reinforcement group (CRe, N = 13), and Positive reinforcement group (PosRe, N = 9). Significant differences are indicated by (*)p < 0.1, *p ≤ 0.05, **p < 0.01, ***p < 0.001. Note that in 4A, the significant results are shown on group level for all three groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

group showed higher scores compared to the CRe group (P = 0.021; Fig. 5). There were also differences for the *Sociality* trait (F(2, 25) = 5.03, P = 0.015), i.e. the NegRe group showed lower scores than the CRe group (P = 0.015; Fig. 5). Lastly, the trait *Inquisitive* differed among training style groups (F(2, 25) = 5.63, P = 0.010), where the NegRe group showed higher scores compared to both the CRe group (P = 0.009) and the PosRe group (P = 0.033; Fig. 5).

4. Discussion

The main aim of this study was to investigate, from the horse's perspective, whether there is a bond between the horse and its owner by recording attachment-related behaviours (e.g. proximity seeking and increased exploration in the presence of the owner compared to a stranger) and separation related distress in the owners' absence using a modified SSP. Moreover, we aimed to test whether training style had an effect on the horses' behavioural and physiological responses during the SSP. Our results showed that the horses spent most time in door proximity during separation and sought human proximity upon reunion, irrespective of whether it was the owner or the stranger. Furthermore, the horses' heart rates increased significantly when separated from the human followed by a decrease in heart rate during reunion, suggesting that horses were distressed when left alone and that the presence of a human attenuated the effect upon reunion. Thus, the horses showed at least two features of attachment, namely separation-related distress and safe heaven as reflected in heart rates. However, the results related to proximity seeking and the secure-base effect were unclear and might, if existing, differ between dyads of different training styles.

In order to trigger attachment-related behaviours the individual needs to be stressed (Bowlby, 1982). We simulated a potentially stressful event by firstly placing the horse in a partly unfamiliar fenced off experimental arena and, secondly, by leaving the horse alone in this environment. Since horses are social prey animals living in groups it is likely that the horses in this study would have perceived the separation as a slightly negative and stressful experience. Even if horses are trained to be alone they still respond with, e.g. higher heart rate when socially separated from conspecifics (Hartmann et al., 2011), which was also reflected in the separation phase in our study by increased heart rates and high number of snores. Snores are short, raspy inhalation sounds associated with low alert situations (Stomp et al., 2018) and were, therefore, likely a response to the separation. Also, there was little difference in locomotion behaviour between the separation and reunion phases that could have caused the change in heart rate in our study. Thus, we propose that the separation phase had been perceived as a stressful event for the horses in our study although it was not expressed in attachment-related behaviours.

There are four behaviours indicative of an attachment relationship between a child and its parent (Bowlby, 1982), and dogs have been shown to cover all four (Rehn and Keeling, 2016). Hence, dogs become stressed during separation from their owner, and they seek proximity and comfort upon reunion, indicating a safe haven effect. In addition, dogs repeatedly reveal behaviours such as increased play and exploratory behaviour together with their owner indicating the secure base effect. To our knowledge, this has not been documented in horses.

Investigating the safe haven effect in this study revealed that the heart rate of the horses decreased upon reunion with both the owner and stranger, and horses also decreased the time they spent in proximity to the door. Hence, what deviates from attachment theory is that both the owner and stranger attenuated the effect of the stressful event and were perceived as safe haven to the horse. The similar response to the owner and stranger reunion resembles results from a recent study by Ijichi et al. (2018). Ijichi et al. (2018) studied stress responses of horses during novel handling procedures (i.e. crossing a tarpaulin, walking through a frame with plastic streamers) while led by their owners and a stranger. They found no difference in the horses' performance (crossing time), in behaviour (e.g. proactive behaviour such as backing away, rearing or standing still) or in physiological responses (heart rate, eye temperature) with regard to handler familiarity. Thus, Ijichi et al. (2018) concluded that an unknown handler can be equally effective as the owner in influencing horses' responses when exposed to potentially stressful situations. In our study, the horses were able to move freely during and after the stressful separation, which may be a better approach when studying attachment-related behaviours instead of leading the horse in a halter. Horse handling in general may facilitate habituation as signals from the handler can overshadow any natural behavioural responses related to, e.g. fear.

The horses in this study sought human proximity upon reunion, which is an important indicative attachment-related behaviour (Bowlby,

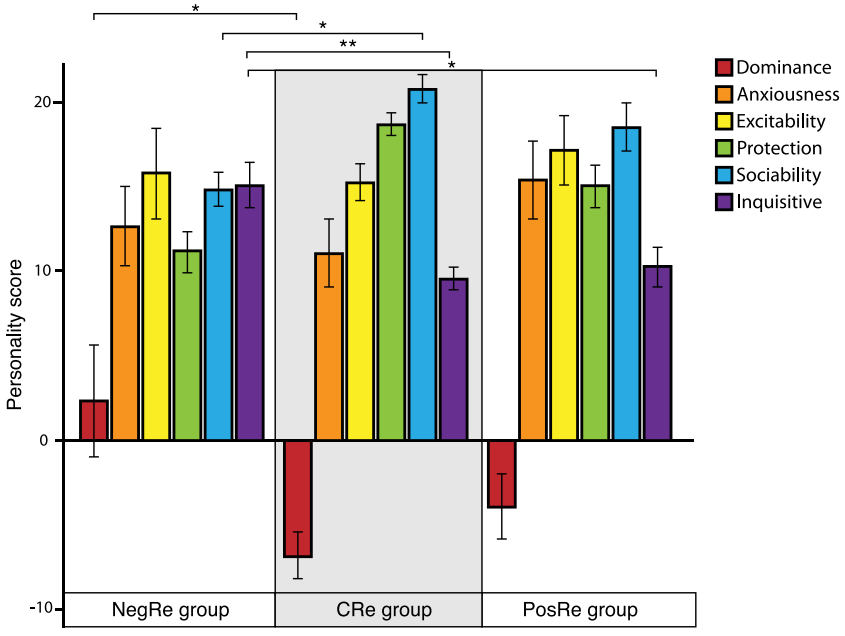

Fig. 5. Personality scores for horses in the Negative reinforcement group (NegRe), Combined reinforcement group (CRe) and Positive reinforcement group (PosRe). Significant differences between groups after pairwise comparisons are indicated by *p < 0.05, **p < 0.01.

1982). Interestingly, the horses spent more time in stranger proximity than in owner proximity during reunion and it was the PosRe training group that was the source for this difference. No difference was found between owner and stranger proximity for the NegRe and CRe group. Owner proximity durations were similar for all training groups during reunion. Our results are contrary to studies in dogs where dogs trained with PosRe seek more contact with the owner than the stranger during both reunion (Vieira de Castro et al., 2019) and during training situations (Deldalle and Gaunet, 2014) compared to dogs trained with NegRe. Similar to our results though, dogs trained with aversive methods are less likely to interact with strangers (Rooney and Cowan, 2011).

Previously, training method has been shown to affect horses' behavioural responses towards humans (Sankey et al., 2010a) where those trained with PosRe approach humans more quickly and also stay longer in human proximity than those trained with NegRe. In addition, Sankey et al. (2010c) found that horses that were rewarded with food were quicker to approach and spent more time with humans than those only rewarded by three strokes of withers' scratching, indicating that the type of reward during PosRe could also influence the behaviour of the horse. For example, there are certainly individual differences in how tactile contact is perceived or, equally, how food is more reinforcing for some individuals than others (Ellis and Greening, 2016). However, the studies by Sankey et al. (2010a, c) only investigated the horses' responses towards the familiar experimenter and did not compare the responses to unfamiliar humans. Still, their results might be comparable to the horses' behaviour towards the stranger in our study. A limitation with the comparisons between training groups in our study is the small number of dyads in each group, and especially in the NegRe group. Nevertheless, we do believe that we captured the owners' main training method with the questionnaire and the training validation test. For future studies, we suggest to recruit a sufficient number of dyads representative of each training approach. Another speculation about the proximity-seeking behaviour towards the stranger could be that horses in the PosRe group have positive experiences of humans and/or that they extensively seek the possibility of receiving a treat (Sankey et al., 2010c). The NegRe training group contained horses that were considered as the most dominant by their owners, but, notably, they were also considered as more inquisitive compared to horses in the PosRe group. It might be a confounding effect of self-reporting via questionnaires and also an effect where people preferring different training methods might have different expectations of the personality of their horse or vise versa, i.e. owners of one type of horse might prefer one type of training method.

Investigating the secure base effect, the horses in our study showed more exploratory behaviour together with the owner than with the stranger during the initial walking and standing still phases. However, one confounding effect of the modified SSP used in this study could be that we always started with the owner. The decrease in exploratory and alert behaviour in the beginning of the stranger experiment compared to the owner experiment might therefore be due to a decrease of novelty of the experimental arena and set-up. During reunion, there was a tendency for the horses to stay longer in door proximity during the stranger reunion compared to the owner reunion, but also to stay more in stranger proximity than owner proximity. The longer distance to the owner could be an indication of a secure base effect with the owner. However, there was no significant difference found for exploratory behaviours between owner and stranger reunion which makes it difficult to speculate further about a secure base effect of the owner from these results. In dogs, the order of the different phases in the SSP is considered important. Rehn et al. (2013) thoroughly investigated a counterbalanced SSP with dogs and found significant order effects for exploration behaviours. Hence, it is suggested to emphasise the behavioural analysis on the reunion phase (Rehn et al., 2013; Rehn and Keeling, 2016; Vieira de Castro et al., 2019). Therefore, greater attention should be placed on the contact-seeking behaviour with the familiar person which, in human attachment theory, is an important aspect of attachment (Bowlby, 1958 and 1982). Still, a larger sample size and a counterbalanced design would be preferred in future studies for improved evaluation of exploratory behaviours. Yet, according to our study, the secure base effect might not be present between horses and their owners. For future studies, it might be interesting to include information about owner's adult attachment style since that has been suggested to have an impact on the behaviour in dogs (Rehn et al., 2017) and horses as recently shown by Arrazola and Merkies (2020). Another explanation for our results could be that, perhaps, the experimental arena was too barren for the horses and that the set-up was not considered novel enough to maintain the horses' interest in exploring. Alternatively, as Rehn and Keeling (2016) proposed, we might not have used the correct behavioural indicators that mirror the attachment system between the animal and its owner. We did find that ear flicking increased more when horses were reunited with the owner and since ears and facial expression are shown to be of great importance in horse-horse communication and expressing emotional states (Wathan and McComb, 2014; Dalla Costa et al., 2014), a more detailed analysis of the alterations in the horses' facial expressions could be interesting to include in future studies.

In conclusion, our results suggest that horses show some attachment-related behaviours towards humans. Interestingly, focusing on the reunion, horses showed stress-relieved behaviours (i.e. less time in door proximity) and decreased heart rate in the presence of both the owner and stranger suggesting a safe-haven effect irrespective of familiarity of the human. The horses also showed exploratory behaviours to a similar degree during the owner and stranger reunion, but differed in door- and stranger proximity between training groups. Hence, we did not find a clear secure base effect. Nevertheless, we would like to encourage more research in this under-explored area and emphasise that future studies should address the daily time owners spend with their horses and include information about training methods used, the owner attachment style, and how much contact horses have to companions given the importance of touch both during horse training and conspecific bonding.

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