# Looking forward to human interactions?

- Horses' anticipatory behaviour as an indication of emotional states in a human-horse training context

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Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of Applied Animal Science and Welfare
Master's program in Animal Science
Uppsala 2024

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|---|---|
| **Credits:** | 30 credits |
| **Level:** | Advanced, A2E |
| **Course title:** | Independent project in Animal Science |
| **Course code:** | EX0870 |
| **Programme/education:** | Master's program in Animal Science |
| **Course coordinating dept:** | Department of Applied Animal Science and Welfare |
| **Place of publication:** | Uppsala |
| **Year of publication:** | 2024 |
| **Cover picture:** | **Heta Rautiainen** |

**Keywords:** *anticipation, classical conditioning, horse emotion, horse-human interaction*



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## Abstract

Expectation for future events, anticipation, is formed through predictable conditioned events and emotional memories. Learning quickly which events have led to positive or negative outcomes, the horse is for example able to seek favourable resources and avoid danger which favours the survival of horses. In the context of human-horse interaction, horses can communicate through contact seeking or avoidance which events with human they have experienced positively or negatively.

This pilot study aimed to find out if the four treatments would lead to differences in anticipatory behaviours that could be used to interpret underlying emotional states. This was done by classically conditioned signals that predicted four different treatments (two positive and two negative). In contrast to the expectations, there was no statistically significant differences in behaviour during the anticipation periods which could suggest some emotional overlap between treatments. Despite the statistical insignificance, some behaviours might suggest positive and negative association to humans and could be used in future studies. We discuss then more the advances and limitations of this test protocol as well as the practical implications of the results for horse-human interactions.

*Keywords: anticipation, classical conditioning, horse emotion, horse-human interaction*

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# Abbreviations

| | |
|---|---|
| BPM | Beats per minute (heart rate) |
| CS | Conditioned stimulus |
| HR | Heart rate |
| HRV | Heart rate variability |
| US | Unconditioned stimulus |

# Introduction

## Emotions

### Definitions of emotion and mood

Emotional reactions are responses to rewarding and pleasant, or aversive, punishing situations and they have adaptive value for individuals (Mendl et al. 2010). These reactions can be distinguished based on their emotional arousal level (high vs. low) and emotional valence (negative vs. positive; Mendl et al. 2010). Furthermore, such emotional reactions are commonly associated with physiological and behavioural changes as well as changes in cognitive processes (Burman et al. 2008; Mendl et al. 2010). Thus, emotions reflect how animals experience a certain situation and can give insight into the animal's state of welfare (Fraser 2008; Mellor et al. 2020). Assessing emotions at the human-animal interface can give important insights into the consequences of human interventions on animal behaviour, emotions, and ultimately welfare (Mellor et al. 2020).

Emotions and further processing of information involve many interconnected cognitive processes. The emotion-eliciting situation initiates activation or inactivation of physiological, emotional, and cognitive processes, which also guide an animal's behavioural response aiming for the most profitable outcome (Spruijt et al. 2001). The quality of emotion and behavioural response is affected by whether the individual animal perceives the situation as positive or negative (Boissy & Erhard 2014). Physiological indicators are used alongside behaviours in measuring and interpreting animal emotions (Hall et al. 2018). For example, cardiovascular responses like heart rate (HR) and heart rate variability (HRV), as well as body temperature, and hormonal responses like cortisol from blood, saliva, or feces, have been commonly used in measuring arousal level in horses (Hall et al. 2018). At the moment, there are not many reliable physiological indicators of emotional valence in horses but oxytocin has been linked to positive emotions (Lansade et al. 2018).

The physiological and behavioural responses are affected by each other during emotional regulation and the formation of psychological and physical outcomes (Mikkelsen et al. 2021).

Emotions and mood can be described by their duration, intensity, and quality. Emotional reactions are short-living responses to situations or events and can be distinguished based on their emotional arousal level (high vs. low) and emotional valence (negative vs. positive) (Mendl et al. 2010). Emotional reactions can be elicited through internal and external stimuli, like hunger or olfactory sensations, respectively. In contrast to short-living emotional reactions, moods are defined as prolonged affective states (Nettle & Bateson 2012). According to Nettle & Bateson (2012), mood "arise when negative or positive experience in one context or period alters the individual's threshold for responding to potentially negative or positive events in subsequent contexts or periods". Moods are formed during longer periods, and they do not need specific triggering stimuli experienced by an individual (Russell 2003; Nettle & Bateson 2012). Both emotional reactions and moods set expectations of the situation, and they have adaptive value for the individual (Russell 2003; Mendl et al. 2010; Nettle & Bateson 2012).

## Identified emotions in horses

Horses, like other mammals, can experience multiple emotions which, as mentioned, include the dimensions of arousal and valence.

In his research on "Affective neuroscience", Panksepp (2011) suggested seven emotional systems for mammals: SEEKING, CARE, PLAY, and LUST for positive emotional states, and RAGE, FEAR, and PANIC for negative ones. Some emotions, like fear and anxiety, seem to be interconnected (Forkman et al. 2007). Moreover, in the work of Mendl et al. (2010), the authors provide terms like excited and happy (high arousal, positive valence), relaxed and calm (low arousal, positive valence), sad and depressed (low arousal, negative valence) and fearful and anxious (high arousal and anxious) to describe an emotion and mood. Their two-dimensional model can be used to describe emotions through intensity and quality (Fig. 1).

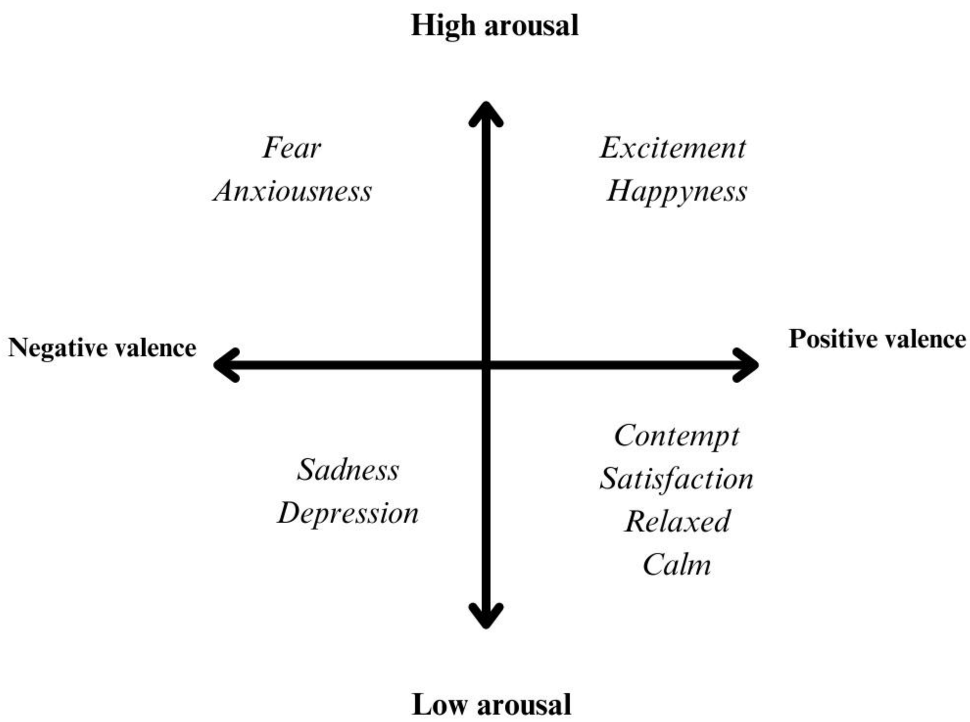


*Figure 1. Modification of the integrative four-dimensional framework of emotion and mood by Mendl et al. (2010). The vertical arrow represents the gradual change between high and low arousal, which is activated and downregulated by the sympathetic and parasympathetic nervous system. The horizontal arrow represents valence, i.e. quality of emotion, which can change between different degrees of positive and negative emotion..*

## The effect of emotions on horse welfare

### Definition of welfare

Welfare is a multidimensional concept where an individual animal's subjective experience related to the environment and the longer-term quality of life is affected by the emotional experiences arising from both physical and mental stimuli (Green & Mellor 2011). The aspects of animal welfare can be divided into three interconnected categories: biological functioning and health, natural living, and affective states (Fraser et al. 1997). To deepen the insight of the impact of different categories on animal welfare, Mellor et al. (2020) proposed that four domains, consisting of nutrition, environment, health, and behaviour (including human-animal-interactions) go through the fifth domain of affective states which produces the experience of welfare. The welfare outcome can vary on a continuum between good and bad. Even though there are many aspects of welfare, it is clear that emotions and mood are inseparable from the animal's subjective experience of its welfare state and other cognitive processes (Fraser 2008; Hausberger et al. 2019). In addition to minimizing negative emotions and merely avoiding suffering, animals' quality of life should be improved also by promoting both short-term and long-term positive experiences (Boissy et al. 2007).

## The importance of positive emotions

Experiencing positive emotions is important for horses since the quality of emotions affects the subjective experience of welfare.

Even though short occasional negative emotions, like hunger, have a biological role, it is generally accepted that good welfare includes experiencing positive emotions over the long term and in multiple aspects of life (Hausberger et al. 2019). To achieve good welfare, in addition to minimizing negative emotions and merely avoiding suffering, an animal's quality of life should be improved by promoting long-term positive experiences (Boissy et al. 2007). These can include opportunities for allogrooming, seeking, play and exploration that have an important biological role in survival and maintaining social relationships (Krueger et al. 2021). Good physical health is also expected to contribute to a positive experience (Mellor et al. 2020). On the contrary, restricted and barren housing conditions, compromised health, and aversive handling may lead to negative mental states and suffering. In the context of human-horse interactions, enhancing predictability and controllability is also important to minimize negative experiences (Carroll et al. 2022).

Understanding equine emotion and cognition is necessary for welfare-focused horse training and husbandry. Domestic horses are subjected to human environments where reliable measurements of emotions, especially positive ones, are needed to assess the welfare of horses in different contexts. Therefore, identifying more reliable indicators of animal emotion would aid the assessment of welfare.

## Human-animal interactions and horse welfare

The quality of human-animal interactions, which relates especially to the aspect of affective states impacts the welfare of horses (Hausberger et al. 2019; Mellor et al. 2020). An important part of welfare and safety in human-horse interactions is building of positive relationship, which is affected by multiple factors in the past and present. Studies suggest that the quality of the contact, as well as the frequency and time of contact with owners and handlers can affect the development of positive relationships (Hausberger et al. 2008; Liehrmann et al. 2022). On the other hand, flight responses arising from negative affective states are connected to injuries of horse handlers regardless of the experience level of the handler (Hausberger et al. 2008; Hawson et al. 2010; Górecka-Bruzda et al. 2015).

Domestic horses have often multiple daily contact with different humans and their welfare is affected by the handling they are receiving (Hausberger et al. 2019; Mellor et al. 2020). In these interactions, routine handling and health care, veterinary procedures, and training for multiple leisure and professional purposes should be assessed from the horse's perspective to avoid unnecessary negative experiences, and to promote positive emotions.

Stress-related behavior from physical and mental suffering can be seen in equestrian sports (König von Borstel et al. 2017), and horses can perceive human handling as negative. On the other hand, interactions like grooming, are known to have a calming effect on a horse (Lansade et al. 2018). As a result of individual interactions, horses can experience a temporary increase or decrease in experience of welfare, but when repeated it can have a much longer lasting impact. Fearfulness toward humans or previous aversive handling experiences could cause unnecessary negative stress to horses when they are forced into contact with humans.

Gaining more insight into emotion-related responses in horses gives an advantage to noticing changes in behaviour well in time and managing interactions in a way that minimizes negative experiences and promotes positive ones.

## Physiological indicators

### *Heart rate*

Cardiovascular responses like HR and HRV have been commonly used in measuring horses' arousal (Hall et al. 2018). The benefit of physiological indicators is that they can be objectively measured and quantified.

Heart rate is a much-used non-invasive measure indicative of high and low arousal in animals. The normal heart rate of an adult horse is 28-40 beats per minute (bpm), and 80-120 bpm in foals. HR can be detected and measured in real-time by a monitor and elastic strap around the horse's chest (over withers and under the belly) (Squibb et al. 2018). Many available HR belts can be used with or without other equipment, like a saddle or trotting harness and represent an effective non-evaisve method for data collection.

HR, like many other physiological measures, has been considered a good indicator of emotional arousal, but unspecific for emotional valence (Briefer et al. 2015; Baciadonna et al. 2020). Therefore, HR measures fit well in combination together with behavioural measures to make a judgment about emotional valence (Hall et al. 2018). However, in some cases, HR can reveal a strong reaction to stressors despite very subtle behavioural signs (Squibb et al. 2018).

Changes in HR may also occur in situations eliciting positive emotions. Positively excitable situations, like the expectation of coming food can increase HR significantly compared to a resting state, although in some individuals increased HR may be a sign of frustration (Peters et al. 2012). On the contrary, positively calming situations, like allogrooming or scratching, have been connected to decreased HR (Feh & de Mazières 1993; Lansade et al. 2018). Furthermore, Thorbergson et al. (2016) found no difference in HR between humans scratching from withers or patting a horse to the neck directly after riding. In summary, the evidence shows that scratching has practical potential to induce positive human-animal interactions.

## Behavioural indicators

In addition to physiological changes, changes in emotion can be seen as behavioural changes of different degrees and functions. Behavioural measures of different affective states of horses include combined or separate analysis of facial expressions, body posture and movement, and vocalizations that can be measured by their duration, frequency, and intensity (Dalla Costa et al. 2014; Gleerup et al. 2015; Wathan et al. 2015; Hall et al. 2018; Torcivia & McDonnell 2021). The repertoire of horses' behavioural and facial expressions is very wide (Christensen et al. 2002; Wathan et al. 2015). Measuring behaviour is a non-invasive assessment of a horse's emotion although it requires a well-defined ethogram and training of the observers to reach good intra- and inter-observer reliability (Hall et al. 2018). Behaviours should be interpreted concerning the context and the whole-body language.

### *Indicators of positive emotions in human-horse interactions*

Positive emotions, often described as joy and calmness, in horses, are connected to fulfilling behavioral needs such as the need for social contact with conspecifics (Krueger et al. 2021). Studies studying positive emotions are few and they often investigate horses' behaviour during human interactions, like grooming, or lack of abnormal behaviours like aggression and stereotypies.

Scratching is found to induce positively valenced emotions. It has been linked to a low head position, relaxation of facial muscles, contact seeking with the handler, and lack of avoidance behaviors (Thorbergson et al. 2016; Lansade et al. 2018). These findings have been supported by physiological measures such as increased blood oxytocin levels, a potential marker for positive emotions (Lansade et al. 2018). Voluntary contact seeking and proximity to even unknown humans have also been suggested to be a sign of positive experience with humans (Lundberg et al. 2020; Larssen & Roth 2022). Horses might also show some laterality in information processing, for example, turning the right ear or right eye towards the source of positive stimuli, like hearing the vocalization of a familiar horse (Basile et al. 2009; Smith et al. 2016). Peters et al. (2012) reported nickering appearing during the waiting for positive events, like feeding. Snorts through nostrils have been recorded both in expectedly positive situations (Stomp et al. 2018) as well as after arousal (Scopa et al. 2018).

### *Indicators of negative emotions in human-horse interactions*

Behavioural responses related to negative affective states, like fear pain, apathy, and frustration can arise from situations often related to fear, pain, or another type of physical or psychological discomfort. The factors causing these states include ethologically suboptimal environments, naturally threatening stimuli, fear conditioning to a stimulus, use of punishments, and use of equipment or aids in a way that causes discomfort. These responses include both active (proactive) and passive (reactive) coping mechanisms for adapting to the current environment (Budzyńska 2014).

Fear has been categorized as high arousal, and negative valence (Mendl et al. 2010). Fear responses have been categorized as fight (active defense), flight (escape), freeze (passive avoidance), and faint (thanatosis). During human-horse interactions, like riding or handling, horses use the same behavioral responses to avoid or attack humans as they would react to an environmental stimulus or social conflict (McGreevy et al. 2009). Some behaviours and expressions connected to acute negative emotions are high neck, avoidance of the stimulus, startle response, and presence of aggressive behaviours (Young et al. 2012). Facial expressions linked to fear include eye white showing, flared nostrils, and attention focusing on the stimulus (Young et al. 2012; Lundblad et al. 2021). Vigilance is also a well-known sign of fear and anxiety in horses (Forkman et al. 2007)**.** In addition, in the study by Smith et al. (2016), horses reacted to pictures of angry faces by left eye lateralization combined with a faster increase in HR which supports the view of different lateralization during opposite emotional contexts.

While the stronger active conflict responses are more easily recognized by people, more passive responses and ease of handling do not equal to low arousal or positive emotion. Squibb et al. (2018) showed in their study that horses can experience fear and show high heart rates despite showing very little behavioural resistance to human handling in the novel surface task.

Frustration is a negative emotion and occurs when a behaviour or situation has not led to an expected positive outcome, thus turning the initial positive emotion into a negative one (Bremhorst et al. 2019). For example, Zupan et al. (2020) reported that a longer than expected waiting time during feeding led to restlessness and pawing in horses, which are signs of frustration.

Tail swishing, reefing on rains, and licking and chewing were detected after riding during neck patting suggesting frustration or a feeling of ambiguity, on the other hand, low head position was detected significantly more often during neck scratches (Thorbergson et al. 2016).

# Anticipation

## Definition and description of anticipation

Anticipation has been described as appraising an upcoming event that has been previously linked to specific emotional reactions and adjusting behaviour according to the arising expectations (Spruijt et al. 2001; Mendl et al. 2010; Anderson et al. 2020). Furthermore, anticipation has been described to be goal-directed, meaning it is linked to waiting for something appetitive, a positive event, but it can also be used to describe an animal's expectation before aversive events (Spruijt et al. 2001; Imfeld-Mueller et al. 2011). Predictability is an important part of arising anticipation and the capacity to adjust behaviour to the situation. Through learning, certain events and stimuli can become predictable and the quality of previous events can be asessed through anticipatory behaviour (Krebs et al. 2017). In positive situations, for example, acquiring of a reward would stop anticipation (Krebs et al. 2017).

During anticipation, animals expect either rewards or punishers that are not yet present but signalled via a classically conditioned stimulus (Anderson et al. 2020). The conditioned stimulus can be, for example, visual, tactile, olfactory, or auditory, and can derive from an animal's environment or a signal produced by a human. Learning the association between the conditioned stimulus (CS) and the unconditional stimulus (US) requires also awareness of both CS and US (Clark & Squire 1998). Expectation of positive events may lead to an animal seeking the appetitive outcome, and on the other hand, to avoid possible aversive events (Anderson et al. 2020).

## Anticipation and animal welfare

Observing how animals express anticipation in different contexts can be one tool to assess their welfare in real-time and give insight into animals' past experiences.

Anticipation of positive events helps animals to seek resources which can be seen as a satisfying experience for themselves (Spruijit et al. 2001). Anticipation of negative events on the other hand helps the animal to avoid unpleasant situations which can lead to a feeling of control (Spruijit et al. 2001; Carroll et al. 2022). Generally, conditioned events including certain times, locations and signals become predictable cue for upcoming event and can be anticipated by animal (Imfeld-Mueller et al. 2011; Jensen et al. 2013; Bremhorst et al. 2019; Baciadonna et al. 2020). Thus, characterization of different anticipatory behaviours would help in the recognition of underlying emotions during human-horse interactions. This, in turn, would allow for modification of handling and training routines that facilitate positive emotions and prevent negative emotions to improve animal welfare.

## Anticipation studies in non-human animal species

Though species-specific differences may occur in the expression of behaviour, previous research on anticipation in multiple species gives a good overview of behavioural and physiological responses that could be linked to different future-oriented emotions. Studies in, for example, silver foxes (*Vulpes vulpes*) (Moe et al. 2006), pigs (*Sus scrofa*) (Imfeld-Mueller et al. 2011), dogs (*Canis lupus familiaris*) (Bremhorst et al. 2019) and goats (*Capra aegagrus hircus*) (Baciadonna et al. 2020) have characterized anticipation as changes in behavioural activity and increased heart rate. All the studies used food to create positive anticipation (Imfeld-Mueller et al. 2011; Bremhorst et al. 2019; Baciadonna et al. 2020) and inaccessible food (Bremhorst et al. 2019; Baciadonna et al. 2020) or a potentially frightening sensory stimulus (Moe et al. 2006; Imfeld-Mueller et al. 2011) to create negative anticipation. An increase in activity has especially been linked to positive anticipation (Peters et al. 2012; Baciadonna et al. 2020). Silver foxes and pigs showed approach behaviour towards the anticipated reward (Moe et al. 2006; Imfeld-Mueller et al. 2011; Peters et al. 2012), whereas they showed signs of avoidance when expecting a negative outcome (Moe et al. 2006; Imfeld-Mueller et al. 2011).

In a study with silver foxes, positive anticipation was measured after conditioning signals for food-related and non-food-related rewards, and negative anticipation was measured after conditioning for grabbing with a neck tong (Moe et al. 2006). In this study, foxes oriented themselves toward the experimenter when anticipating positive rewards, and in contrast, increased their distance from the experimenter when anticipating negative events.

Baciadonna et al. (2020) tested positive (food), mild negative (inaccessible food), and neutral (clicker) anticipation with goats. The goats were trained for a delay between CS and US from 20 s to 5 min gradually for 11 days. The authors found that both heart rate and the number of behavioural responses, like activity, rapid head movements, calls, and ears pointing forwards, increased in expectation of positive conditions, suggesting higher emotional arousal compared to other conditions. The authors also found that asymmetric or horizontal ear positions were connected most to negative treatments.

Imfeld-Mueller et al. (2011) measured the anticipation of pigs under positive (access to popcorn) and negative (crossing a partially black ramp) conditions. The authors found that avoidance, such as latency to move and turn around in the test area, was higher during the negative conditions and suggested to be a good indicator of negative emotional valence. Moreover, the study also found that the heart rate did not differ in different conditions.

Bremhorst et al. (2019) studied the facial expressions of dogs as indicators of positive (access to food) and negative (access to food blocked) anticipation. In the study, ears were found to be flattened more in the negative conditions and adducted more in positive conditions, whereas nose lick and panting were found to be rare in positive conditions (ibid.?). The authors found that other expressions more common in negative conditions were blinking, parted lips, and jaw drop.

Zimmermann et al. (2011) measured anticipation of domestic fowl (*Gallus gallus domesticus*) to a positive (mealworms), negative (water spray), and neutral event (no consequence). In this study, three different auditory cues were used to indicate the upcoming events. the test arena consisted of a closed experimental enclosure with a push door separating the starting point and the reward bowl (Zimmermann et al. 2011). The authors found that fowls showed more head movements and stepping, and less foraging during negative anticipation compared to positive and neutral anticipation. The authors also found an increase in comfort behaviours during positive anticipation compared to the other conditions.

In a study with bottlenose dolphins, dolphins showed high anticipation after a conditioned whistle signal, and anticipation was a good predictor of engagement in following positive human-animal interaction or self-play with toys (Clegg et al. 2018). Atlantic bottlenose dolphins also showed increased alertness towards handlers and the surroundings after cues that predicted the starting of the public show. Thus, anticipation was shown by decreased general activity and waiting near the show area (Jensen et al. 2013). In these two studies, the authors concluded that anticipation could predict engagement in upcoming events.

## Anticipation studies in domestic horses (*Equus caballus*)

Like other species, horses learn to associate events to previously neutral stimuli by classical conditioning (McLean & Christensen 2017). They also have a rich variety of facial and behavioral expressions for different emotions (Young et al. 2012; Wathan et al. 2015; Hall et al. 2018; Lansade et al. 2018).

Previously, two studies (Peters et al. 2012; Zupan et al. 2020) have investigated anticipatory behaviour in horses related to upcoming feeding, finding similarities with other species. Peters et al. (2012) characterized the equine anticipatory response, i.e., behaviour and heart rate, to a food reward. The delay from CS to the arrival of food was increased from 10 s to 10 min. They found that during anticipation, the general activity (standing, locomotion, arousal, and investigation) as well as HR increased compared to baseline.

In the study by Zupan (2020), horses engaged in comfort behaviours, looked to the door, kicked, and pawed both at the predicted feeding time and significantly more when the predicted feeding time was delayed one hour without gradual training. Earlier feeding time than expected decreased anticipatory behaviour, such as looking at the direction of the door and spending more time resting than in other treatments (ibid.).

Bohák et al. (2018) had contradictory results on measuring possible pre-race anticipatory cortisol and HRV responses in harness racing horses and it was expected horses would have been conditioned to race cues by themselves.
Studying anticipation would be a promising tool to address this need for new information about horse's perceptions in the context of human-horse interactions, like training.

## Methods of measuring anticipation

Anticipation can be studied by measuring behavioural and/or physiological reactions to rewards, punishers, or neutral events that are not yet present but signaled via a classically conditioned stimulus (Andresson et al. 2020). Positive outcomes have included food (Peters et al. 2012) and play (Clegg et al. 2018), or the arrival of enrichment (Clegg et al. 2018). Negative outcomes have included blocking access to food (Bremhorst et al. 2019; Baciadonna et al. 2020), crossing visually challenging surfaces (Imfeld-Mueller et al. 2011), water spray (Zimmerman et al. 2011) or physical induced pain (Moe et al. 2006). Auditory stimuli, like music (Zimmerman et al. 2011), or a combination of whistle and two clicks (Baciadonna et al. 2020), are some examples of CS used in previous studies. Some studies have trained a delay between CS and US varying from a few seconds to minutes (Peters et al. 2012; Baciadonna et al. 2020).

Training Approaches for conditioning animals to positive and negative anticipation have varied between studies. After introducing CSs separately on different days, Zimmermann et al. (2011) used also a mixture of positive, negative, and neutral CS to be sure fowls had learned to discriminate between different CS before testing started. On the other hand, Imfeld-Mueller et al. (2011) tested positive and negative anticipation on the same pigs in separate trials. In the study by Baciadonna et al. (2020), goats were conditioned for only one treatment (positive, negative, or neutral).

Studies have measured anticipation in either separate experimental enclosure (Imfeld-Mueller et al. 2011; Bremhorst et al. 2019; Baciadonna et al. 2020) or in the animal's home enclosure (Moe et al. 2006; Peters et al. 2012; Clegg et al. 2018; Zupan et al. 2020). Possible doors, signals, and outcomes have been operated mostly without physical and visual contact between animal and experimenter (Imfeld-Mueller et al. 2011; Baciadonna et al. 2020), except silver fox (Moe et al. 2006), bottlenose dolphins (Clegg et al. 2018), dogs (Bremhorst et al. 2019) and horses (Peters et al. 2012; Zupan et al. 2020) where experimenters or owners have been involved with holding animals or providing the positive or negative outcome.

There seems not to be a direct test designed for assessing anticipatory behaviour in a training context with humans.

AIM & HYPOTHESES

In previous studies, anticipation has been studied in different species in the context of management routines and during human-animal interactions. Studies on horses have shown anticipatory behaviour during feeding routines but not in human-horse interactions. Learning more about anticipation in horses could help interpret horse behaviour during emotionally conditioned situations and help adjust handling and training practices.

This pilot study aimed to develop a pilot anticipation test for human-horse interactions and fill this knowledge gap, aiming to characterize behavioural indicators representing underlying emotional states in horses expecting positive and negative valences interactions with humans.

We hypothesise that horses would show different behavioural signs depending on the classically conditioned treatment (high and low arousal, positive and negative valence).

*How does horses' behaviour change during anticipation of different events (eliciting high/low arousal and positive/negative valence)?*

# Method

## Animals and housing

Six horses from Säva Ridcenter in Uppsala, Sweden, were used for this study. Horses were riding school horses, housed individually in boxes, and turned out in the field in groups. Horses were participating in riding school activities during the study period. Inclusion criteria for horses were that i) horses should be healthy with no visible signs of sickness and not under veterinary care, ii) have been at least 2 months at the riding school. Sex, age, breed, or training level were not considered during the selection of horses.

Six horses were selected for the training and testing of the anticipation protocol. Two horses (one Swedish warmblood gelding 14 years old and one Irish cob mare 17 years old) were used for the pre-experimental phase where the training protocol was tested, and data from these two horses were not included in the analysis. Four horses (2 mares, and 2 geldings aged 10-22 years) were used for the experimental phase and for testing of anticipation (Table 1). During the training and testing period, all horses were kept on summer pasture during daytime and were stabled in individual boxes during nighttime. The training and data collection were performed from mid-July to mid-August of 2021.

*Table 1. Overview of horses including breed, sex, and age of horses that were included in testing and data collection.*

| Horse | Breed | Sex | Age |
|---|---|---|---|
| 1 | Welsh pony | Gelding | 16 |
| 2 | Irish cob | Mare | 10 |
| 3 | Swedish warmblood | Mare | 12 |
| 4 | Shetland pony | Gelding | 22 |

## Experimental design

Training and testing were carried out in two paddocks with gravel surface, measuring approximately 13x18 meters each (Figure 2). Contrary to previous studies, the handler, i.e. experimenter was in contact with the horses. The experimenter held the horse on a lead rope attached to a stable halter made of broad nylon while presenting the visual cue and delivering the treatment. The person handling the camera was standing behind the fence.

Horses were trained one at a time while another horse waited in the second, adjacent paddock. Since the mares and geldings were kept in separate pastures, the two mares were taken to training and testing at the same time and so were the two geldings. This allowed for visual contact between familiar horses and to avoid separation anxiety and thus loss of attention during training and testing. In the beginning of the first training session, each horse had the possibility to freely explore the arena for 5 minutes.

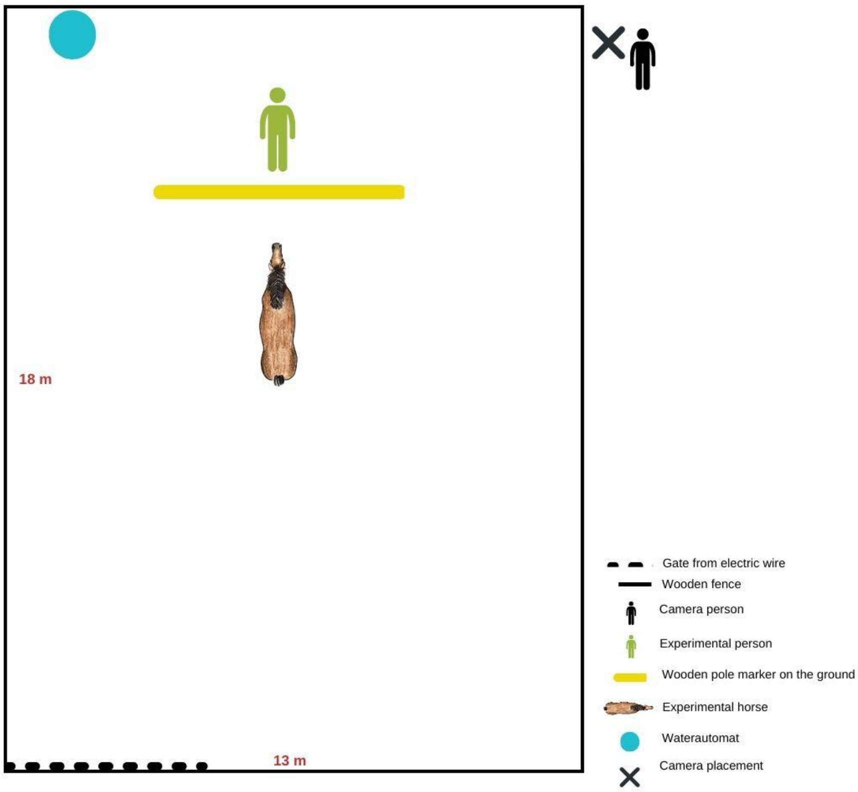


*Figure 2. The layout of the experimental paddock during both training and anticipation testing. The experimenter was standing behind a pole that was marking the position for both human and horse. The experimenter held the horse on normal stable halter and lead rope while presenting the cue and delivering the treatment.*

## Treatments

All horses were exposed to four treatments that aimed to induce different emotions with high or low arousal combined with positive or negative emotional valence (Mendl et al. 2010). Horses were classically conditioned to specific cues, i.e.small objects before each treatment (Table 2). The four objects associated with each treatment were chosen randomly for each horse (Table 3) to minimize the possible effect of the object/CS itself on the behaviour.

For the high arousal positive valence (H+) treatment, the horses received food (carrot) from the experimenter's hand. Food has been found to be a consistently attractive reinforcer which can motivate horses to perform even very physical demanding tasks (Olczak et al. 2018). Previously, food (hay, grains) has been used in two studies looking at anticipatory behaviour in horses (Peters etc. 2012; Zupan etc. 2020). Scratching with the hand at the horse's preferred place on the body was chosen for the low arousal positive valence (L+) treatment. Scratching has been shown to induce relaxed behaviour and an increase in oxytocin in horses during horse-human contact (Hintze et al. 2017; Lansade et al. 2018).

Waving a plastic stripe on a whip was chosen for the high arousal negative valence (H-) treatment because sudden waving objects, like plastic bags, have shown to provoke nervousness in horses (Hintze et al. 2017). Finally, pinching the horse at the neck was chosen as the low arousal negative valence (L-) treatment because it is likely experienced as a very unpleasant feeling. Tightening a girth and pinching the neck were tested with the two pre-test horses and pinching was chosen due to its fast applicability and effectivity on horses. All the three other treatments were also tested with the two pre-experimental horses. In Figure 3, each treatment is represented as part of two-dimensional framework of affective states by Mendl etc. (2010)

*Table 2. The treatments and their descriptions.*

| Treatment | Description of treatment after symbol is presented |
|---|---|
| H+ / High arousal, positive valence | Horse gets food (carrot) from human's hand. |
| L+ / Low arousal, positive valence | Horse receives scratches for 3 seconds from a place that they individually enjoy. |
| H- / High arousal, negative valence | Waving a whip with red and white plastic strips. |
| L- / Low arousal, negative valence | Pinching horse at the neck. |

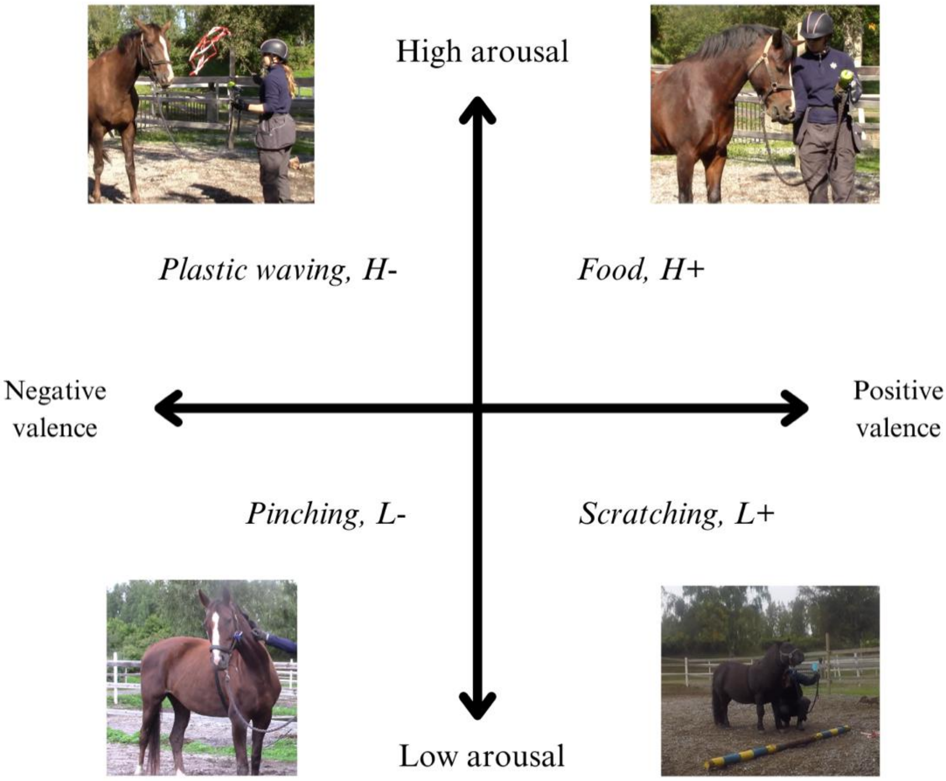


*Figure 3.. Treatments represented as each emotional dimension. Graph modified from Mendl et al.(2010)*

*Table 3. The cues used for each horse during each treatment. The selection of cues for each horse was done randomly.*

| Horse | Treatment | | | |
|---|---|---|---|---|
| | Pinch L- | Food H+ | Scratching H- | Waving H+ |
| 1 | Green frisbee | A green bottle with grey plastic strips attached. | Orange block | Blue target on gray stick |
| 2 | Blue target on gray stick | A green frisbee | Green bottle | Orange block |
| 3 | Green bottle | An orange block | Blue target on gray stick | Green frisbee |
| 4 | Orange block | A blue target on a gray stick | Green frisbee | Green bottle |

## Training phase and protocol

Training of horses was carried out by one person (experimenter) on four separate days at the end of July 2021. The baseline behaviour of each horse was filmed for 5 minutes before the start of each training session. The first two days of training were reserved for creating the association between the cue and the subsequent treatment. Each treatment was applied for four times, and this was repeated five times during the training day. Each repetition was followed by a short break of around one minute. After the 20 repetitions, the horse would receive a break and be provided with hay and water, and another horse was taken in for training.

During the first two days, horses received training for two treatments per day in separate sessions, each lasting around 30 minutes while another horse was trained in between. Day three and four were reserved to training that treatments can be alternated during one session as well as to test the learning criteria (see 3.3.2 Learning criterion). This training followed the same structure of 4 x 5 repetitions before being tested on the learning criteria. However, training was stopped as soon as the horse showed an ability to discriminate between the two treatments. For treatments with positive valence we looked for signs of approach, and on treatments of negative valence we looked for signs of avoidance. The outline of training order of treatments for each horse can be seen in Table 4.

On training days, both the order of horses taken in for training and the order of treatments was balanced to minimize the possible effect of training order on the behaviour. If the horse showed the conditioned response to the symbol presented before being presented with the actual consequence, the training was stopped, and the horse was tested on the learning criteria. If the horse showed signs of aggression or high fear during the presentation of a symbol or while receiving the treatment, the training was stopped for safety reasons. Horses that were not responding to one or more treatments within the first two days would be excluded, however, all horses responded to the treatments and thus none was excluded.

The training took place between 8:00 and 14:00 on each day. During training, the weather was sunny and warm (25-35 °C).

*Table 4. The outline of training days (1-4). On day 1 and day 2, it is shown which horses received training for which treatments and in which order. Day 3 and 4 treatments were alternated for each horse and on those days learning criteria was tested on said treatments.*

| | Treatment | | | |
|---|---|---|---|---|
| Horse | Day 1. Training | Day 2. Training | Day 3. Mixing and learning criteria | Day 4. Mixing and learning criteria |
| 1 | H+, L- | H-, L+ | L-/H- | L+/H+ |
| 2 | L+, H- | L-, H+ | L+/L- | H-/H+ |
| 3 | H-, H+ | L+, L- | H+/L+ | L-/H- |
| 4 | L-, L+ | H+, L- | H-/H+ | L+/L- |

### Learning criterion

The criteria for learning were tested on days 3 and 4 (Table 4) after horses showed associative learning between the symbol (conditioned stimulus) and the subsequent treatment. The testing of the learning criteria was done during six consecutive repetitions per treatment, one treatment at a time. The conditioned stimulus was presented for 3 seconds before introducing the treatment. When the horse consistently showed signs of approach (e.g., walking or reaching towards human) or avoidance (stepping back, turning head), towards the conditioned stimulus before receiving the treatment, it was assumed that the horse had learned the association between the stimulus and the treatment, i.e. it is anticipating the treatment. After confirmation of a formed association, the horse could move on to the anticipation testing phase. If the horse did not fulfil the learning criterion, i.e. it would receive more training consisting of 5 repetitions at a time to form a better association between the symbol and the following treatment. In this study, all horses passed the learning criterion on first try.

## Testing of anticipation

The testing of anticipation included recording baseline behaviour and reminding the horse of the treatment, and finally the anticipation test itself. To film the baseline behaviour and record baseline heart rate, the horse was allowed to explore the test arena freely during 5 minutes before testing started. Both people involved in testing stood outside of the arena during these first 5 minutes, after which the experimenter went into the paddock and led the horse to the testing spot (Fig. 2). The camera person stood outside of the arena, filming and letting the experimental person know when to start and stop the 10-second anticipation period. Before testing, reminding of treatment cues was done by showing the horse the conditioned cue and immediately delivering the treatment. The experimental design can be seen in figure 2. Like during training, one horse was tested at a time, while the other horse was waiting in the second paddock. The heart rate belt and monitor were fitted when horse was led to the test arena, before recording of baseline data.

### Recording

During testing of anticipation, behavioural data was collected by using two cameras (GoPro Hero 5 and Canon Legria), and heart rate was collected by using Polar V800 watch paired with Polar H6 Bluetooth smart heart rate sensor on a girth around the horse's chest. The same paired watch and sensor was used for all horses. The girth and sensor were fitted around the horse, with the sensor on the left side near the armpit. Skin/hair under the sensor was wetted with water, also electrogel (Cefar Blågel, Malmö) was used. The watch was attached to the girth near withers.

### Anticipation testing protocol

Anticipation testing was divided over two consecutive days. During each test day, all horses were tested for anticipation in two out of the total four treatments. As testing took place two weeks after the training, the test session started with three reminder repetitions of each treatment. During these repetitions, the conditioned stimulus was immediately followed by the treatment. This was made to ensure that the horse remembered the cues and treatments used. After the reminder repetitions, the horse was led in a small circle away and back to the testing spot, to get a small break before testing commenced. Thereafter, all six 10-second anticipation periods (i.e., 3 periods per treatment) were conducted in a balanced order after one another. A schematic illustration of the anticipation testing order can be seen in Figure 4. The testing protocol followed the training in that two horses were brought to the test arena at a time, where one horse was tested while the other horse waited in the second paddock to avoid stress from social isolation.

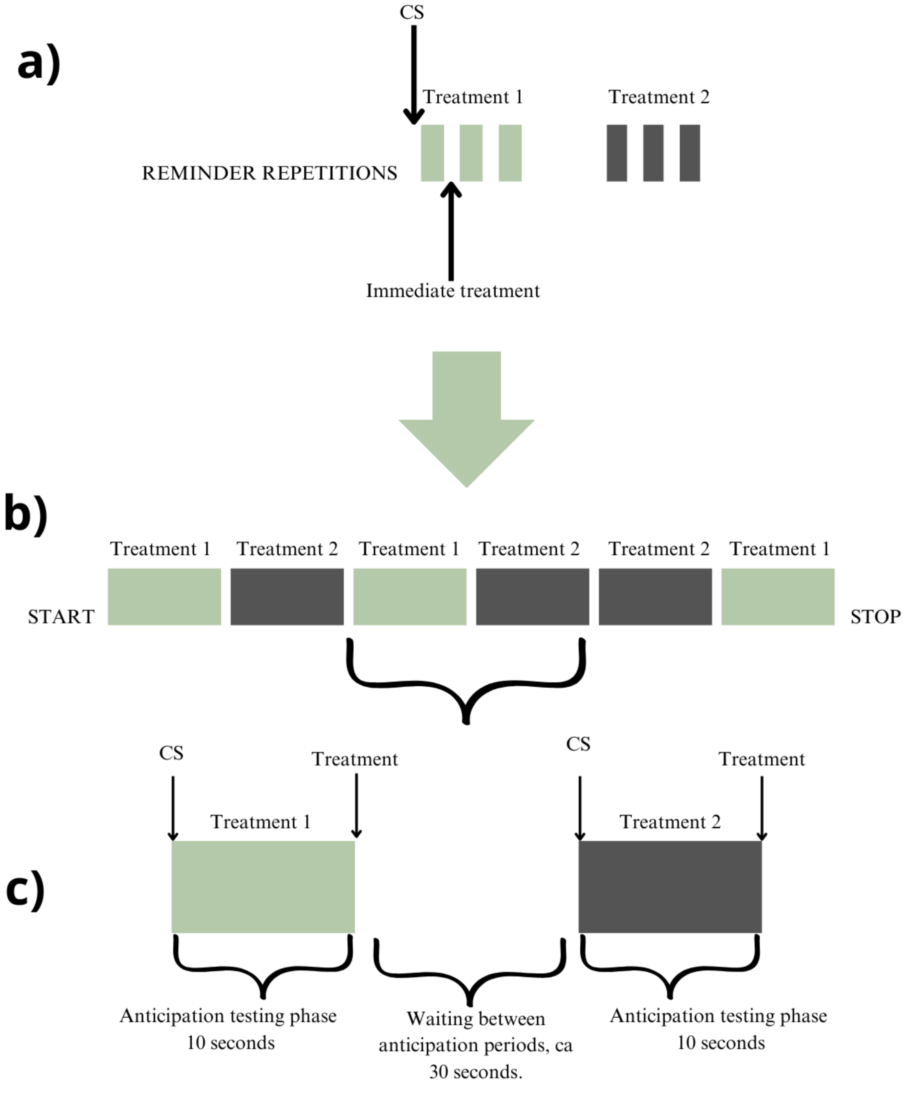


*Figure 4. The example outlines of anticipation testing. Two different treatments (represented by green and grey boxes) were tested at a time in a balanced order. CS = classically conditioned stimulus, specific cue for each treatment. At a), 3 reminder repetitions of the presentation of cue and treatment were made before the actual testing of anticipation. At b) is an example of a random order of two different treatments. At c) is a detailed illustration of each repetition: introduction of the cue, anticipation time, delivery of treatment, and 30-second break before the next repetition.*

*Table 4. Testing anticipation of 2 treatments per day (day 5 and 6 of the whole protocol including training). Each day is marked with the combination of treatments which was tested on each horse respectively.*

| Horse | Day 5Day 6 | |
|---|---|---|
| | Day 5 | Day 6 |
| 1 | H-/L+ | H+/L- |
| 2 | H+/L+ | H-/L- |
| 3 | H+/L- | H-/L+ |
| 4 | H-/L- | H+/L+ |

# Analysis

## Video coding

All 3x10 second anticipation periods from each treatment for each horse were included in the analysis. Video footage of baseline behaviour and reminder repetitions were excluded from the analysis. Behaviours during the anticipation testing were coded according to the ethogram in Table 5, using the Behaviour Observation Research Interaction Software BORIS (Friard & Gamba 2016). Behaviours were coded as point events in BORIS. Frequency of all behaviours was recorded from a stopped video by instantaneous sampling by marking which behaviours occurred on each time point (start of each second) during the 10 second anticipation period and was later summarized in Microsoft Excel. The Ethogram was constructed based on previous studies on assessing emotions in horses and was first evaluated with the two pre-experimental phase horses.

*Table 5. Ethogram used for analysis of anticipation based on behaviours defined in previous studies.*

| Behavior | Description |
|---|---|
| **Ears forward** | Both ears are turned or swiwelled forward (rostrally) (Wathan et al. 2015; Lansade et al. 2018) |
| **Ears backwards** | Both ears are rotated laterally and dorsally/caudally. The opening of the inner ear is turned outwards (Wathan et al. 2015; Lansade et al. 2018) |
| **Ears axially** | "Ears straight to the side, perpendicular to the head-rump axis."(Wathan et al. 2015; Lansade et al. 2018) |
| **Contact with the handler** | "Horse's upper lip is extended and mobile, horse nibbles the handler or any other element in front of it (wall, leading rein, etc.)."(Lansade et al. 2018) |
| **Chew (non-nutritional)** | "Distances the lower jaw from upper jaw in the vertical or oblique direction, being able to perform a vertical or circulatory movement and rapidly decreases the distance between the lower jaw of the upper jaw, approaching the upper and lower teeth. This behaviour can be performed with the mouth open or closed. This movement may or may not occur together with licking."(Trindade et al. 2020) |
| **Stands still** | Stands with all 4 legs on the ground quite straight under the body without taking steps back or forth or sideways.(Zupan et al. 2020) |
| **Three legs supporting body** | Three legs supporting the body weight and one hind leg without load. (Trindade et al. 2020) |
| **Leaning towards human** | Leaning to human by shifting weight to front of the body and front legs. Hoofs/legs not straight under the body, hind legs left stretched backwards. |
| **Forward moving** | Moves at least once with two legs forward, resulting in new position. (Trindade et al. 2020) |
| **Backward moving** | Moves at least once with two legs backward resulting in new position. (Trindade et al. 2020) |
| **Sideways moving (right/left)** | Moves at least once with two legs sideways, resulting in new position. (Trindade et al. 2020) |
| **Stomp** | "Suddenly flexing and then extending a limb, sharply striking the hoof against the substrate" (Peters et al. 2012; Trindade et al. 2020) |
| **Head turning away** | "Keeps the head towards one side (left or right) with a flexed neck, looking to a stimulus somewhere in the environment, including the observer, with one or both ears oriented forward. The horse remains a few seconds in this position |

| | |
|---|---|
| | before returning the head in the longitudinal axis of its body (distinguishing between left and right head turn).”(Trindade et al. 2020) |
| **High neck** | Head and neck raised above wither level, angle between neck and withers of 130 to 165 °. (Lansade et al. 2018) |
| **Low neck** | Upper neckline and head are below the wither level. Angle between neck and withers of 202 to 237°. (Lansade et al. 2018) |
| **Neutral neck position** | Upper neckline and head are approximately same height as wither level. Angle between neck and withers of 166 to 201°. (Lansade et al. 2018) |
| **Tail neutral** | Upper part of tail rests relaxed against body, relaxed straight down. (Young et al. 2012) |
| **Tail swish** | “Moves its tail to the left and right above the line groin or moves it up at least 45 degrees and down quickly.“ (Trindade et al. 2020) |
| **Sniffs ground** | Head and neck down below wither level, muzzle within 2 cm from the ground. |
| **Investigate cue** | Sniffing or nibbling object that is used as a cue for treatment. |
| **Unscorable** | Body part cannot be seen and scored. E.g., out of camera view or behind other body part. |
| **Pawing** | “One foreleg is lifted off the ground and extended forward followed by a dragging backward movement with the hoof digging the ground.” (Peters et al. 2012; Trindade et al. 2020) |
| **Sniffing human** | Sniffing the handler on whatever part of body or clothes. |

### Data editing and statistical analysis

Results from the video analysis with BORIS were extracted to Microsoft Excel. The amount of all behaviours during each treatment on each horse were summarized in numeric form. The mean frequency of each behaviour during the 30-second anticipation period (3x10 seconds per treatment) was then calculated. There were 14 unscorable behaviour points where the part of horse was hidden (horse 1: 1 behaviour “mouth” in food treatment; horse 2: 1 behaviour “mouth” in food treatment, 1x behaviour “mouth” and 5x behaviour “ears” on scratch treatment, 6x behaviour “tail” on pinch treatment). The effects of unscorable and missing data was corrected before statistical analysis. During calculating mean frequencies of all behaviours in all treatments and horses, the number of unscorable behaviours were deducted from the sum of that behaviour class that unscorable behaviour belongs to (for example, mouth or ears).

Frequencies and means per 30 seconds of all behaviours for each horse and treatment were calculated in Microsoft Excel. Thereafter, Friedman test was run in Minitab (version 19.2020.1.0, year 2020). From Friedman test, one behaviour that was closest to statistical significance ($p < 0,05$) was chosen for further analysis using Wilcoxon test.

Heart rate recordings were not reliable due to a lot of missing data and therefore this data was excluded from analysis.

# Results

All four horses were tested for all four treatments. The behavioural results from statistical analysis are explained in their own sections and discussed further.

## Learning association between US and CS

Training of 20 repetitions of 1 treatment took around 15-20 min per horse per day, including breaks. All horses learned to associate the cue for the specific treatment and passed the learning criteria for the mixing of signals within 3-4 days before testing anticipation.

## Horse-human interactions

Figure 5 shows the mean frequency (per 30 seconds) of different horse-human interactions (i.e., sniffing human, leaning towards human, investigate the cue) during all anticipation periods (3 x 10 sec) per treatment. All horses showed signs of interaction with the human. The anticipation of waving of the plastic bag resulted in the least number of horse-human interactions (mean= 0). The highest frequency of horse-human interactions was seen during anticipation of food (sniffing human mean= 0,25; leaning towards human mean=0,32; investigate cue mean =0,15). The interaction with the human was recorded less frequently during anticipation for pinching (mean=0,075; 0,1; 0,03, respectively) and scratching (mean= 0,08; 0,08; 0,05, respectively) than during anticipation for food treatment.

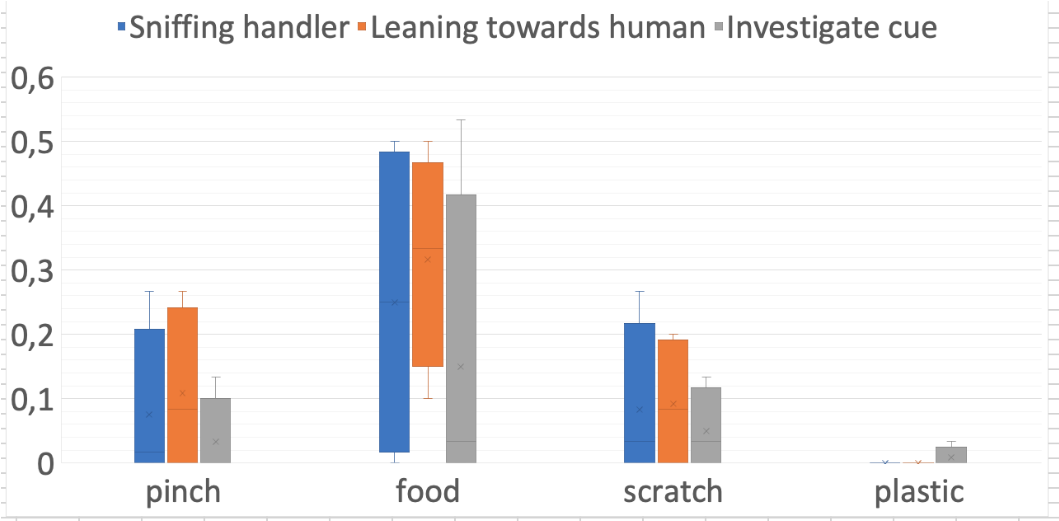


*Figure 5. Horse-human interactions during anticipation of different treatments. The graph shows mean frequencies per 30 second period from test periods with all horses. Most human directed interactions were seen during food treatment and least during waving of the plastic strips (plastic in figure).*

## Head and neck position

Overall, there was no statistically significant difference in head-neck positions (i.e., head turned left or right, high neck, low neck and neutral neck position) (Figure 6) between the four treatments. Neutral neck position was observed on average most frequently during anticipation of all treatments (pinching mean= 0,8; food mean= 0,5; scratch mean= 0,6; plastic mean= 0,8) compared to other head and neck positions (head turning, high neck, and low neck). Turning head away was seen more frequently during the anticipation for pinching (mean= 0,4) and waving of plastic strips (mean= 0,5) compared to anticipation of food (mean= 0,1) and scratching (mean= 0,2).

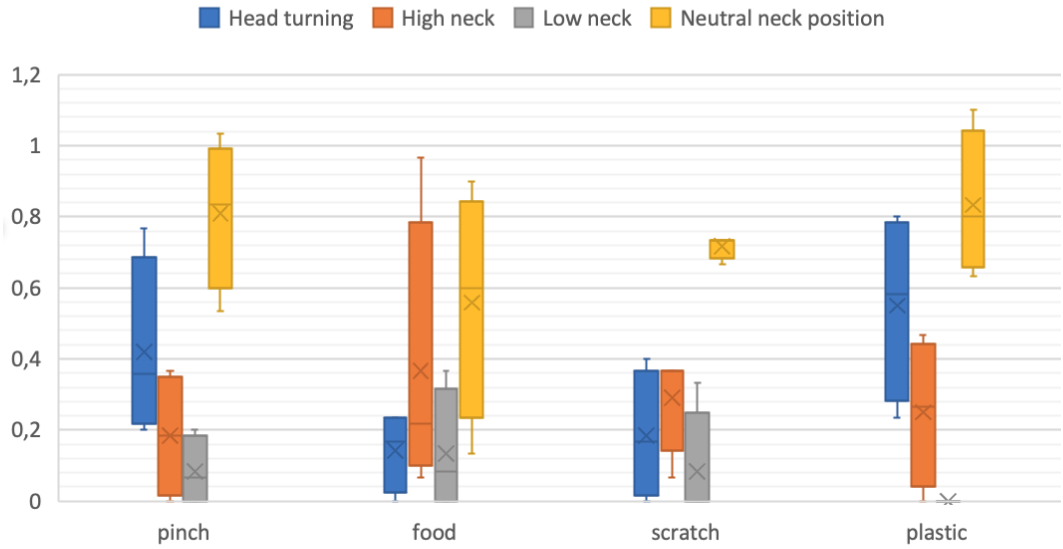


*Figure 6. The mean frequency of head and neck movements during all four different treatments. Blue: turning of the head. Orange: High neck. Grey: Low neck. Yellow: Neutral neck position.*

From all behaviours, only the mean frequency of "head turning" was shown to be close to statistically significant difference (p= 0,045, Friedman test) between treatments, thus it was chosen for further analysis with Wilcoxon test. No other behaviour was affected by treatment.

Wilcoxon test showed no statistically significant effect when comparing the mean values of head turning between each treatment (Table 6). The largest difference between mean frequency on head turning was between treatment 1 (pinching) and treatment 4 (waving of plastic strips) (p-value 0,059).

*Table 6. Results of behaviour "head turning" based on the Wilcoxon test. The effect of treatments on anticipatory behaviour were compared to each other. Treatments: 1 (pinching on neck), 2 (food), 3 (scratching) and 4 (waving of plastic strips).*

| Treatment | median diff | p-value |
|---|---|---|
| 1-2 | -0,5 | 0.25 |
| 1-3 | -0,5 | 0.116 |
| 1-4 | -0,5 | 0.059 |
| 2-3 | 0 | 1 |
| 2-4 | 0 | 0.371 |
| 3-4 | 0 | 0.371 |

## Ear position

Overall, there was no statistically significant difference between different treatments in the frequency of ear positions (i.e., ears forward, ears backwards, ears axially). However, the frequencies of the ear movements varied similarly in every treatment (Figure 7). Horses spent more time having ears forward or axially during all anticipation periods than ears backwards. The ears forward was observed more during anticipation for pinching (mean= 0,6) and anticipation for food (mean=0,6) than ears axially (mean= 0,4; 0,4). Ears forward and ears axially had also greater variability from very low to high frequencies during the anticipation periods. Ears backward was seen less frequently (mean values: pinching=0,025; food= 0,04; scratch=0,08; plastic=0,05) and it had less variation between treatments.

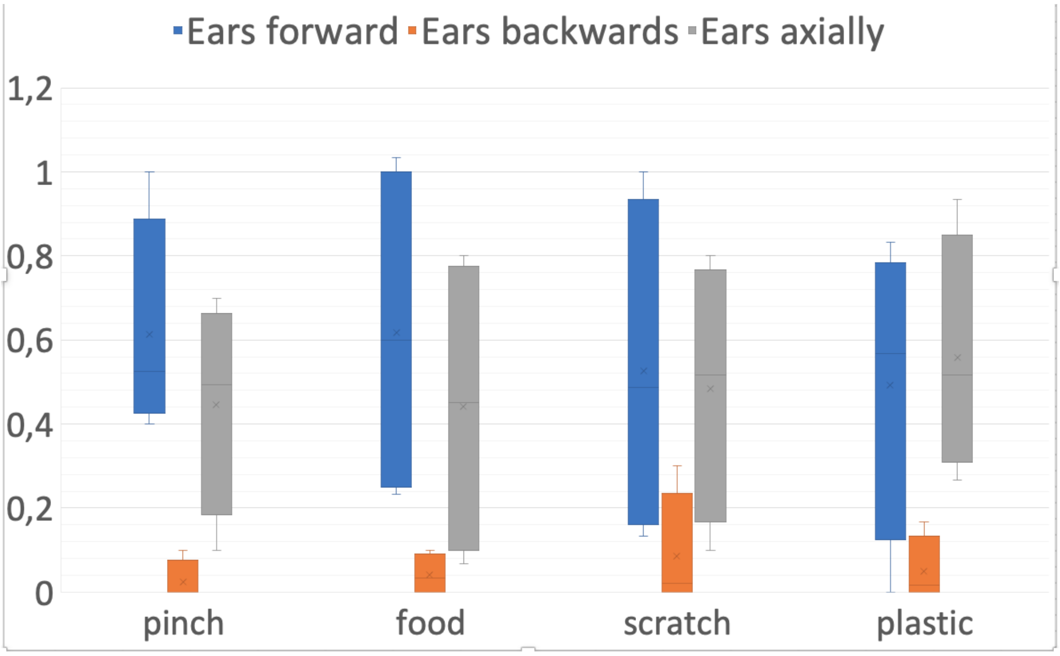


*Figure 7. Ear movements during anticipation. Horses spent least time ear backwards in all treatment conditions. "Ears forward" was most frequently observed during anticipation of all treatments.*

# Discussion

The pilot study aimed to find out if the four treatments would lead to differences in anticipatory behaviours that could be used to interpret underlying emotional states. The results showed that horses exhibited similar behaviours through all anticipation periods and no statistically significant effects were found between treatments. This could be related to the nature of the study (pilot study) and the small number of horses used. Other reasons could be related to the method and suggestions for future studies will be discussed in more details below.

### *Study design*

One reason for the insignificant effect on behaviours could be that the anticipation periods were not separated well enough and thus caused emotional overlap. Deviating from previous studies on horses (Peters et al. 2012; Zupan et al. 2020) this study tested a mixture of two CS-US combinations instead of testing one conditioned signal at a time. Even though horses were able to learn the association between the cue and the outcome, mixing the treatments in the test situation could have produced a carryover effect (Mendl et al. 2010) where previous emotion is overriding the following event. For example, when mixing anticipation for food and waving of plastic strips, one horse showed a strong motivation to approach the human even though the handler showed the cue for the upcoming negative treatment. In addition to inattentiveness or passive coping mechanisms, another reason for few behavioural expressions observed especially during the negative treatments could be habituation to the US (e.g., waving of plastic straps).

### *Horse-human interactions and practical relevance*

Even though this study failed to find clear indicators related to anticipation and emotional states, these findings could have practical relevance in horse training. The results showed highest mean frequency of horse-human interactions during anticipation for food compared to anticipation of other treatments, and therefore the use of food could be recommended for future anticipation studies on horse-human interactions. However, it can only be concluded that by increased activity, contact seeking and making physical contact, the horses might merely express wanting the food. Therefore, this data of increased activity during the anticipation period might not us to draw the conclusion that these behaviours are an expression of horses experiencing positive valence. It is also possible that some horses were even expressing frustration for withholding the reward (the 10 second CS-US-delay in testing phase) compared to continuous rewarding (the 1-3 second CS-US delay in training phase) or the behaviours could be result of unintentional learning. The study by Zupan et al. (2020) also observed increased activity when routine feeding time was delayed by one hour. Even though positive emotions were present in the beginning of the anticipation period, a review by Anderson et al. (2020) argues that it would not be possible to judge from instantaneous sampling which was used in this study. Instead, the same authors also suggest the use of physiological and neurological measures but due to technical issues, the heart rate data collected for this study was not usable.

There was on average no positive trend on seeing more horse-human interactions during anticipation for scratching compared to anticipating pinching. Least interactions were seen during anticipation of waving of plastic strips. These are in line with previous research suggesting that horses taking contact with humans is a sign of positive association with humans (Lundberg et al. 2020; Larssen & Roth 2022) whereas avoidance behaviours reflect a negative association with humans

(Young et al. 2012). Only the behaviour “head turning” varied most between treatments which could also be a potential sign of avoidance behaviour. Even though the difference between treatments was not statistically significant, turning the head away from the human was seen more frequently during the anticipation of the two negative treatments (pinching and waving of plastic strips) and in contrast was seen very infrequently during anticipation of the positive treatments (food and scratching).

Some observations, like standing still during the mixing of positive and negative treatments could be interpreted as avoidance or behavioural shut down. This could support the avoidance of combining rewards and punishments in horse training. Although undetectable via statistical analysis, the video footage from this study showed that the horses stood often motionless when mixing positive and negative treatments. Some horses can express their discomfort by minimizing their behavioural expression (passive coping style) (Squibb et al. 2018). More detailed analysis of facial expressions could be added to look for small signs of discomfort or relaxation instead of looking for more big gross behavioural signals. This could be justified since horses have a wide range of small behavioural signals of discomfort that would benefit to be observed by technology like EquiFACS (Wathan et al. 2015).

### *Future studies*

Future studies should aim to establish an experimental set-up where treatments are clearly separated in time to avoid emotional contagion and make them more distinguishable from the horse's perspective.

To start with the benefits of this pilot study, in this setting the horses were oriented towards both the CS and the US which helps the horse to pay attention to events. The training protocol developed was very simple and the training of four horses could be carried out by one person. The anticipation testing was done by the same person and another person was responsible for filming. To be able to carry out a study with a larger number of horses, a team consisting of 2-4 people training and testing horses could be considered.

Some benefits of the experimental setting also came with downsides. The horses were tested in a familiar outdoor paddock and had visual contact with another horse. Thus, horses were never isolated from conspecifics and showed no signs of separation anxiety. However, the paddock was exposed to external environmental stimuli from traffic, and by passers. From video footage it shows that this might have led to horses’ attention being occasionally drawn to external events. Furthermore, horses were exposed to heat (+35 C) which could have led to inattentiveness to the testing situation due to discomfort caused by weather conditions. In previous studies, anticipatory behaviour in horses has been tested in a stable environment indoors (Peters et al. 2012; Zupan et al. 2020). Therefore, future studies on human-horse interaction and anticipation in horses should consider testing horses in other familiar but more stable environments, like an indoor riding hall. In this study tested horses had visual contact to each other which could cause social transmission of emotional states (Rørvang et al. 2018). To minimize social transmission and isolation stress, placing a companion horse that is not participating in the study close to the test arena could be considered. The number of test horses (n=4) was low so a refined test protocol would have to be tested on larger number of horses to validate findings and further improve reliability and generalizability.

To minimize the effect of emotional overlap discussed earlier, future studies should test anticipation for different positive and negative events separately which would give the horse time to recover from the previous event and avoid emotional overlap that could hinder seeing clear differences between the treatments. Separate test events could also support predictability, which has been identified as an important part of formation of anticipatory behaviour (Krebs et al. 2017). From an ethical point of view, separating the training and testing of different emotional states might help minimizing unnecessary use of negative treatments and thereby minimisediscomfort

In this study, the horses appeared to have effectively learned the treatments when presented individually. However, in the testing phase, it was difficult to see clear reactions to the two treatments classified as "low arousal" (pinching and scratching). This could mean horse's reactions could have been misinterpreted as learning even if their behaviours were simply reactions to anticipation rather than the treatments themselves. In the future, the behavioural criteria for learning the association between the cue and the subsequent treatment should be revised to minimize misinterpretation but also to stop the training of the negative CS-US pairing to cease as soon as learning is achieved.

### *Consideration of ethical, societal and sustainability aspects*

Horses are used for multiple purpose for human's recreation. They are sentient beings who's welfare is impacted by human management where they can be exposed to both positive and negative events (Hausberger et al. 2019). Even though strong negative emotion elicited behaviours can be a risk for safety, horses bring many benefits for humans, including social company, rehabilitation, labour, food, fame and economic benefits (Hausberger et al. 2007; Lönker et al. 2020). Yet, it is under ethical discussion if horses benefit from these interactions and what practices are morally justified (Heleski 2023). Moreover, from a sustainability perspective, concentrating only on the benefits horses bring to humans is very anthropocentric. A more interspecies approach to sustainability, which centers on the horse's experience is needed for discussions on the ethics of horse-human interactions (Bergman 2019). This perspective could also be extended to the use of horses in research, like in this study, emphasizing the importance of considering the horse's lived experience and welfare in study design and methodology.

The owners of the horses acquired for this study were informed about the four treatments (two positive and two negative) and they voluntarily choose to take part. No personal information of the horse owners was collected, and invasive measures were taken. Thus, an ethical permit for animal experimentation was not obtained for this study, however expected harms and benefits should be discussed in relation to different ethical theories.

The justification for this study, which involves exposing horses to discomfort is based on a mix of utilitarian and animal rights perspectives in ethics (Röcklinsberg et al. 2017), i.e., some harm with upper limits is allowed to achieve possible benefits for advancing existing knowledge on anticipatory behaviour in horses and providing insights to assist horse owners and improve horse welfare. The utilitarian perspective seeks to ensure that the benefits of the actions compensate for possible harms caused to the participants. Applied from Jeremy Bentham's point of view on utilitarianism (Röcklinsberg et al. 2017), wellbeing and needs of both horses and humans should be considered equally when assessing the benefits and harms. This study partly violates horse's basic needs since horses are separated from their social group and they are exposed to at least mild fear and pain. The study imposed at least short-lasting discomfort to the horses. The familiar horse pairs were separated for 1-2 hours per day from their group at the pasture and taken to small, separate gravel paddocks with water and hay. This restriction of social contact and grazing could impact the welfare but in this context the treatments could be estimated to have bigger impact. The training and testing took approximately 30-40 minutes per horse per day. The negative treatments included exposing horses to a level of fear and pain that is expected to be mild to moderate when compared to the *severity classifications of animal experiments from the European Directive 63/2010/EU* (Table 3.2 at Vieira de Castro et al. 2017). The negative treatments compromise welfare at least temporarily and it is not known if the horses in this study would have generalized fearfulness to other situations, e.g. interactions with riding school students. While the direct benefits of the horses in this study are not very obvious, there is potential for a larger long-term benefit. This pilot study might have benefited the experimental horses directly through the positive conditioning through food and scratching but it is not known if it is enough to compensate for the negative treatment. Wellbeing was considered by providing free hay and water during breaks, a familiar horse on the other side of the fence and through positive reinforcement training. The Expected outcomes of a revised protocol and further educational material could facilitate behavioural changes in humans and enhance horse welfare during human interactions. This information would help horse handlers in recognizing the emotions horses associate with human presence or tack, helping them to avoid situations that make horses uncomfortable and expose humans to safety risks. It would also support the promotion of positive experiences and initiate re-training when necessary.

For studies using aversive stimuli, the most applicable aspects of 3R compliance are reduction and refinement (Vieira de Castro et al. 2017). To To apply Reduction, studies involving negative stimuli should use the minimal number of horses needed for statistically valid analysis of data. The number of horses used in studies could also be reduced by re-using data from existing studies to identify signs of anticipatory behaviour, e.g. studies from horse training that include positive or negative classical conditioning.

Refinement could be achieved by updating the protocol and minimizing any potential harm. Only smallest effective amount of discomfort or fear to achieve the study's objectives should be used. Horses could express their discomfort in various and subtle ways which requires continuous observation to allow early intervention.

Potential new solutions could include gaining data on anticipatory behaviour from real-life observations where learning is potentially involved (e.g., flees when seeing a saddle, expects rewards in training, or appearance of familiar caretaker on other than feeding times) could reduce the need to expose more horses to time consuming training and testing protocols. The horse's reaction to different stimuli (e.g., equipment) could be filmed in an environment where the horse has the choice to approach or avoid the person and equipment, and the video is analysed but the horse is not being forcefully exposed to the stimulus. The observations could be complemented with interviewing horse owners to collect information on horse's handling history and clinical status.

According to very strict views on animal rights (Röcklinsberg et al. 2017) and interspecies sustainability (Bergman 2019), use of horses in experimental purpose despite the beneficial outcomes from the study. Both views argue that animals are autonomous beings and have moral rights to be unharmed independent of what others want from animals. The feminist ethics of care does not categorically ban interactions with animals (Röcklinsberg et al. 2017). Strict view would suggest similar outcome with animal rights theory where no instrumental use of animals is allowed and suggests that any state and intervension from their natural being is worse for their welfare. However, it could be derived from ethics of care that positive conditioning protocols not based on pre-study deprivation of needs could add up to the lives of the domestic animals. Many ethical dilemmas regarding animal use in research concentrate on negative outcomes on animals. Justification of using animals in studies that have expected outcome for positive experience is not widely discussed. From the interspecies sustainability aspect, voluntary through positive interactions would also support the horse's own agency. Therefore, studying only anticipation from positively emotional situations would bring many benefits also to the horses and could be more easily justified.

# Conclusion

Horses did not show distinct behavioural expressions related to different treatments maybe due to mixing treatments of different valence and arousal levels. Instead, horses could benefit from testing one emotional eliciting event at a time.

Future studies could investigate expressions during human-horse interactions not yet addressed in previous anticipation studies on horses, such as making contact with human, subtle facial expressions linked to ears, eyes and mouth, as well as differentiating between direction of movements. Refining of test protocols in future studies should consider at least clearer signs (CS), only one CS/US pair, and more a training and testing environment with fewer external stimuli.

# References

Anderson, C., Von Keyserlingk, M. A. G., Lidfors, L. M., & Weary, D. M. (2020). Anticipatory behaviour in animals: A critical review. *Animal Welfare*, *29*(3), 231-238. https://doi.org/10.7120/09627286.29.3.231

Baciadonna, L., Briefer, E. F., & McElligott, A. G. (2020). Investigation of reward quality-related behaviour as a tool to assess emotions. *Applied animal behaviour science*, *225*, 104968. https://doi.org/10.1016/j.applanim.2020.104968

Basile, M., Boivin, S., Boutin, A., Blois-Heulin, C., Hausberger, M., & Lemasson, A. (2009). Socially dependent auditory laterality in domestic horses (Equus caballus). *Animal cognition*, *12*, 611-619. https://doi.org/10.1007/s10071-009-0220-5

Bergmann I.M. (2019). Interspecies Sustainability to Ensure Animal Protection: Lessons from the Thoroughbred Racing Industry. *Sustainability*. 11(19):5539. https://doi.org/10.3390/su11195539

Bohák, Z., Harnos, A., Joo, K., Szenci, O., & Kovács, L. (2018). Anticipatory response before competition in Standardbred racehorses. *PLoS One*, *13*(8), e0201691. https://doi.org/10.1371/journal.pone.0201691

Boissy, A., & Erhard, H. W. (2014). How studying interactions between animal emotions, cognition, and personality can contribute to improve farm animal welfare. In *Genetics and the behavior of domestic animals* (pp. 95-129). Academic Press. https://doi.org/10.1016/B978-0-323-85752-9.00014-7

Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., ... & Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology & behavior*, *92*(3), 375-397. https://doi.org/10.1016/j.physbeh.2007.02.003

Bremhorst, A., Sutter, N.A., Würbel, H., Mills, D.S. & Riemer, S. (2019). Differences in facial expressions during positive anticipation and frustration in dogs awaiting a reward. *Scientific Reports*, 9 (1), 19312. https://doi.org/10.1038/s41598-019-55714-6

Briefer, E. F., Tettamanti, F., & McElligott, A. G. (2015). Emotions in goats: mapping physiological, behavioural and vocal profiles. *Animal Behaviour*, *99*, 131-143. https://doi.org/10.1016/j.anbehav.2014.11.002

Budzyńska, M. (2014). Stress reactivity and coping in horse adaptation to environment. *Journal of Equine Veterinary Science*, *34*(8), 935-941. https://doi.org/10.1016/j.jevs.2014.05.010

Burman, O. H., Parker, R., Paul, E. S., & Mendl, M. (2008). A spatial judgement task to determine background emotional state in laboratory rats, Rattus norvegicus. *Animal Behaviour*, *76*(3), 801-809. https://doi.org/10.1016/j.anbehav.2008.02.014

Carroll, S.L., Sykes, B.W. & Mills, P.C.C. (2022). Moving toward Fear-Free Husbandry and Veterinary Care for Horses. *Animals*, 12 (21). https://doi.org/10.3390/ani12212907

Christensen, J.W., Zharkikh, T., Ladewig, J. & Yasinetskaya, N. (2002). Social behaviour in stallion groups (Equus przewalskii and Equus caballus) kept under natural and domestic conditions. *Applied Animal Behaviour Science*, 76 (1), 11–20. https://doi.org/10.1016/S0168-1591(01)00208-8

Clark, R. E., & Squire, L. R. (1998). Classical conditioning and brain systems: the role of awareness. *science*, *280*(5360), 77-81. https://doi.org/10.1126/science.280.5360.77

Clegg, I. L., & Delfour, F. (2018). Cognitive judgement bias is associated with frequency of anticipatory behavior in bottlenose dolphins. *Zoo biology*, *37*(2), 67-73. https://doi.org/10.1002/zoo.21400

Dalla Costa, E., Minero, M., Lebelt, D., Stucke, D., Canali, E., & Leach, M. C. (2014). Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine castration. *PLoS one*, *9*(3), e92281. https://doi.org/10.1371/journal.pone.0092281

Feh, C., & De Mazières, J. (1993). Grooming at a preferred site reduces heart rate in horses. *Animal behaviour*, *46*(6), 1191-1194. https://doi.org/10.1006/anbe.1993.1309

Forkman, B., Boissy, A., Meunier-Salaün, M.-C., Canali, E. & Jones, R.B. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Stress and Welfare in Farm Animals*, 92 (3), 340–374. https://doi.org/10.1016/j.physbeh.2007.03.016

Fraser, D. (2008). *Understanding Animal Welfare: The Science in its Cultural Context*; Wiley-Blackwell: Oxford, UK.

Fraser, D., Weary, D. M., Pajor, E. A., & Milligan, B. N. (1997). A scientific conception of animal welfare that reflects ethical concerns. *Animal welfare*, *6*(3), 187-205. doi:10.1017/S0962728600019795

Friard, O. & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, 7 (11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

Gleerup, K. B., Forkman, B., Lindegaard, C., & Andersen, P. H. (2015). An equine pain face. *Veterinary anaesthesia and analgesia*, *42*(1), 103-114. https://doi.org/10.1111/vaa.12212

Górecka-Bruzda, A., Kosińska, I., Jaworski, Z., Jezierski, T., & Murphy, J. (2015). Conflict behavior in elite show jumping and dressage horses. *Journal of Veterinary Behavior*, *10*(2), 137-146. https://doi.org/10.1016/j.jveb.2014.10.004

Green, T. C., & Mellor, D. J. (2011). Extending ideas about animal welfare assessment to include 'quality of life'and related concepts. *New Zealand veterinary journal*, *59*(6), 263-271. https://doi.org/10.1080/00480169.2011.610283

Hall, C., Randle, H., Pearson, G., Preshaw, L. & Waran, N. (2018). Assessing Equine Emotional State. *Applied Animal Behaviour Science*, 205.

https://doi.org/10.1016/j.applanim.2018.03.006

Hausberger, M., Roche, H., Henry, S. & Visser, E.K. (2008). A review of the human–horse relationship. *Applied Animal Behaviour Science*, 109 (1), 1–24. https://doi.org/10.1016/j.applanim.2007.04.015

Hausberger, M., Stomp, M., Sankey, C., Brajon, S., Lunel, C., & Henry, S. (2019). Mutual interactions between cognition and welfare: The horse as an animal model. *Neuroscience & Biobehavioral Reviews*, *107*, 540-559. https://doi.org/10.1016/j.neubiorev.2019.08.022

Hawson, L. A., McLean, A. N., & McGreevy, P. D. (2010). The roles of equine ethology and applied learning theory in horse-related human injuries. *Journal of Veterinary behavior*, *5*(6), 324-338. https://doi.org/10.1016/j.jveb.2010.06.001

Heleski, C. R. (2023). Social license to operate–why public perception matters for horse sport–some personal reflections. *Journal of Equine Veterinary Science*, *124*, 104266.

Hintze, S., Murphy, E., Bachmann, I., Wemelsfelder, F. & Würbel, H. (2017). Qualitative Behaviour Assessment of horses exposed to short-term emotional treatments. *Applied Animal Behaviour Science*, 196, 44–51. https://doi.org/10.1016/j.applanim.2017.06.012

Imfeld-Mueller, S., Van Wezemael, L., Stauffacher, M., Gygax, L., & Hillmann, E. (2011). Do pigs distinguish between situations of different emotional valences during anticipation?. *Applied Animal Behaviour Science*, *131*(3-4), 86-93. https://doi.org/10.1016/j.applanim.2011.02.009

Jensen, A. L. M., Delfour, F., & Carter, T. (2013). Anticipatory behavior in captive bottlenose dolphins (*Tursiops truncatus*): A preliminary study. *Zoo Biology*, *32*(4), 436-444. https://doi.org/10.1002/zoo.21077

Krebs, B. L., Torres, E., Chesney, C., Kantoniemi Moon, V., & Watters, J. V. (2017). Applying behavioral conditioning to identify anticipatory behaviors. *Journal of Applied Animal Welfare Science*, *20*(2), 155-175. https://doi.org/10.1080/10888705.2017.1283225

Krueger, K., Esch, L., Farmer, K. & Marr, I. (2021). Basic Needs in Horses?—A Literature Review. *Animals*, 11 (6). https://doi.org/10.3390/ani11061798

König v. Borstel, U., Visser, E.K. & Hall, C. (2017). Indicators of stress in equitation. *Special Issue on Equitation Science in Practice*, 190, 43–56. https://doi.org/10.1016/j.applanim.2017.02.018

Lansade, L., Nowak, R., Lainé, A.-L., Leterrier, C., Bonneau, C., Parias, C. & Bertin, A. (2018). Facial expression and oxytocin as possible markers of positive emotions in horses. *Scientific Reports*, 8 (1), 14680. https://doi.org/10.1038/s41598-018-32993-z

Larssen, R. & Roth, L.S.V. (2022). Regular positive reinforcement training increases contact-seeking behaviour in horses. *Applied Animal Behaviour Science*, 252, 105651. https://doi.org/10.1016/j.applanim.2022.105651

Liehrmann, O., Viitanen, A., Riihonen, V., Alander, E., Koski, S. E., Lummaa, V., & Lansade, L. (2022). Multiple handlers, several owner changes and short relationship lengths affect horses' responses to novel object tests. *Applied Animal Behaviour*

*Science*, *254*, 105709. https://doi.org/10.1016/j.applanim.2022.105709

Lundberg, P., Hartmann, E. & Roth, L.S.V. (2020). Does training style affect the human-horse relationship? Asking the horse in a separation–reunion experiment with the owner and a stranger. *Applied Animal Behaviour Science*, 233, 105144. https://doi.org/10.1016/j.applanim.2020.105144

Lundblad, J., Rashid, M., Rhodin, M. & Haubro Andersen, P. (2021). Effect of transportation and social isolation on facial expressions of healthy horses. *PLOS ONE*, 16 (6), e0241532. https://doi.org/10.1371/journal.pone.0241532

Lönker NS., Fechner K., Wahed AAE. (2020). Horses as a Crucial Part of One Health. *Vet Sci*. Feb 29;7(1):28. doi: 10.3390/vetsci7010028.

McGreevy, P., Oddie, C., Burton, F.L. & Mclean, A. (2009). The horse-human dyad: Can we align horse training and handling activities with the equid social ethogram? *Veterinary journal (London, England : 1997)*, 181, 12–8. https://doi.org/10.1016/j.tvjl.2009.03.005

McLean, A. N., & Christensen, J. W. (2017). The application of learning theory in horse training. *Applied Animal Behaviour Science*, *190*, 18-27. https://doi.org/10.1016/j.applanim.2017.02.020

Mellor, D.J., Beausoleil, N.J., Littlewood, K.E., McLean, A.N., McGreevy, P.D., Jones, B. & Wilkins, C. (2020). The 2020 Five Domains Model: Including Human–Animal Interactions in Assessments of Animal Welfare. *Animals*, 10(10), 1870. https://doi.org/10.3390/ani10101870

Mendl, M., Burman, O.H.P. & Paul, E.S. (2010). An integrative and functional framework for the study of animal emotion and mood. *Proceedings of the Royal Society B: Biological Sciences*, 277 (1696), 2895–2904. https://doi.org/10.1098/rspb.2010.0303

Mikkelsen, M. B., Tramm, G., Zachariae, R., Gravholt, C. H., & O'Toole, M. S. (2021). A systematic review and meta-analysis of the effect of emotion regulation on cortisol. *Comprehensive Psychoneuroendocrinology*, *5*, 100020. https://doi.org/10.1016/j.cpnec.2020.100020

Moe, R. O., Bakken, M., Kittilsen, S., Kingsley-Smith, H., & Spruijt, B. M. (2006). A note on reward-related behaviour and emotional expressions in farmed silver foxes (Vulpes vulpes)—basis for a novel tool to study animal welfare. *Applied Animal Behaviour Science*, *101*(3-4), 362-368. https://doi.org/10.1016/j.applanim.2006.02.004

Nettle, D. & Bateson, M. (2012). The Evolutionary Origins of Mood and Its Disorders. *Current Biology*, 22 (17), R712–R721. https://doi.org/10.1016/j.cub.2012.06.020

Panksepp, J. (2011). Cross-species affective neuroscience decoding of the primal affective experiences of humans and related animals. *PloS one*, *6*(9), e21236. https://doi.org/10.1371/journal.pone.0021236

Peters, S.M., Bleijenberg, E.H., van Dierendonck, M.C., van der Harst, J.E. & Spruijt, B.M. (2012). Characterization of anticipatory behaviour in domesticated horses (Equus caballus). *Applied Animal Behaviour Science*, 138 (1), 60–69. https://doi.org/10.1016/j.applanim.2012.01.018

Russell, J. A. (2003). Core affect and the psychological construction of emotion. *Psychological review*, *110*(1), 145. https://doi.org/10.1037/0033-295X.110.1.145

Rørvang, M. V., Christensen, J. W., Ladewig, J., & McLean, A. (2018). Social learning in horses—fact or fiction?. *Frontiers in Veterinary Science*, *5*, 212. https://doi.org/10.3389/fvets.2018.00212

Scopa, C., Palagi, E., Sighieri, C. & Baragli, P. (2018). Physiological outcomes of calming behaviors support the resilience hypothesis in horses. *Scientific Reports*, 8. https://doi.org/10.1038/s41598-018-35561-7

Smith, A.V., Proops, L., Grounds, K., Wathan, J. & McComb, K. (2016). Functionally relevant responses to human facial expressions of emotion in the domestic horse (Equus caballus). *Biology letters*, 12 (2), 20150907

Spruijt, B. M., Van den Bos, R., & Pijlman, F. T. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. *Applied Animal Behaviour Science*, *72*(2), 145-171. https://doi.org/10.1016/S0168-1591(00)00204-5

Squibb, K., Griffin, K., Favier, B. & Ijichi, C. (2018). Poker Face: Discrepancies in behaviour and affective states in horses during stressful handling procedures. *Applied Animal Behaviour Science*, 202. https://doi.org/10.1016/j.applanim.2018.02.003

Stomp, M., Leroux, M., Cellier, M., Henry, S., Lemasson, A. & Hausberger, M. (2018). An unexpected acoustic indicator of positive emotions in horses. *PLoS ONE*, 13. https://doi.org/10.1371/journal.pone.0197898

Thorbergson, Z.W., Nielsen, S.G., Beaulieu, R.J. & Doyle, R.E. (2016). Physiological and Behavioral Responses of Horses to Wither Scratching and Patting the Neck When Under Saddle. *Journal of Applied Animal Welfare Science*, 19 (3), 245–259. https://doi.org/10.1080/10888705.2015.1130630

Torcivia, C. & McDonnell, S. (2021). Equine Discomfort Ethogram. *Animals*, 11 (2). https://doi.org/10.3390/ani11020580

Trindade, P.H.E., Hartmann, E., Keeling, L.J., Andersen, P.H., Ferraz, G. de C. & Paranhos da Costa, M.J.R. (2020). Effect of work on body language of ranch horses in Brazil. *PLOS ONE*, 15 (1), e0228130. https://doi.org/10.1371/journal.pone.0228130

Wathan, J., Burrows, A.M., Waller, B.M. & McComb, K. (2015). EquiFACS: The Equine Facial Action Coding System. *PLOS ONE*, 10 (8), e0131738. https://doi.org/10.1371/journal.pone.0131738

Young, T., Creighton, E., Smith, T. & Hosie, C. (2012). A novel scale of behavioural indicators of stress for use with domestic horses. *Applied Animal Behaviour Science*, 140. https://doi.org/10.1016/j.applanim.2012.05.008

Zimmerman, P. H., Buijs, S. A. F., Bolhuis, J. E., & Keeling, L. J. (2011). Behaviour of domestic fowl in anticipation of positive and negative stimuli. *Animal Behaviour*, *81*(3), 569-577. https://doi.org/10.1016/j.anbehav.2010.11.028

Zupan, M., Štuhec, I. & Jordan, D. (2020). The Effect of an Irregular Feeding Schedule on Equine Behavior. *Journal of Applied Animal Welfare Science*, 23 (2), 156–163.

https://doi.org/10.1080/10888705.2019.1663734

# Acknowledgements

I would like to thank my main supervisor Elke Hartmann for their advice and support throughout planning and carrying out this thesis project. I would also thank my assistant supervisors Christa Wyss and Linda Keeling for their insights in the beginning of the project as well as comments on written work. A big thank you as well to my examiner Claes Andreson for taking their time for this thesis!

Thank you for Säva Ridskola who kindly let us use their horses and facilities for the experimental part.

Lastly, I would also like to thank for my friends for support and motivation during finalising the thesis.

# Popular science summary

Expectation for future events, anticipation, is formed through predictable, conditioned, events and emotional memories. Learning quickly which events have led to positive or negative outcomes, the horse is able to seek favourable resources and avoid danger which favours the survival of horses as a species. In the context of human-horse interaction, horse can communicate through contact seeking or avoidance which events with human they have experienced positively or negatively.

From previous studies horses are known to show anticipation during management routines, like feeding time. It is not studied previously how they would express anticipation during human-horse interactions. Therefore, this study aimed to find out which behaviours could represent anticipation of both positively and negatively valenced emotional events during interaction with human handler. We conditioned horses to four different pairs or conditioned stimulus and unconditioned stimulus (two positive and two negative) and then filming their reaction for 10 seconds after presenting the conditioned stimulus.

Our study failed to find any statistically significant differences in behaviour between the four treatments, but this could be merely due to low number of test horses and limitations on study design and does not mean horses cannot anticipate events with humans. The results could mean horses found presentation of several (especially opposite) emotional events confusing. During training phase, all the horses learned successfully the association between each visual cue and treatment when presented individually. Failure to find any difference in testing phase could be explained by our study design where we mixed two treatments at the time.

In future studies, the problems of this study design could be fixed by testing anticipation for different emotional events separately. This could benefit also predictability which is important part of formation of anticipation. For practical relevance, this should be considered in human-horse interactions to avoid presenting positive and negative events simultaneously.

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