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The effect of human interaction on acute and chronic stress in domestic horses

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Bibliography

1   Introduction

The relationship between humans and horses is a story that has lasted for thousands of years and is very complex. Domesticated wild horses (*Equus callabus*) first appeared around 4000 BC and became an integral part of human society. Since then, they have served mankind in many different ways. Starting as milk and meat suppliers, through draft horses and load carriers, to today's sport and leisure partners. With changing times, also the influence of humans on the well-being of the horses has changed. One can observe the increasing number of behavioural disorders in horses and the rising number of accidents related to horses (Hausberger et al., 2008). About a quarter of these are the result of miscommunication between rider and horse (Keeling et al., 2016). Their relationship with each other is therefore an influence that should not be underestimated, which can have a strong impact on the health of horses as well as the safety of humans and horses (Kelly et al., 2021). It is important to recognize and correctly interpret stress indicators in horses to avoid negative effects on their well-being and to increase safety for both (Merkies & Franzin, 2021). A relationship is the result of ongoing interactions that can have a cumulative effect (Kelly et al., 2021). Consistently positive interactions between human and horse can strengthen the relationship with each other and be a benefit to both (Budzynska et al., 2023a). However, human-horse interaction (HHI) is a major scientific challenge due to its diverse elements. Already the variety of purposes, the variations in personalities of humans and horses, differences in breeds, rearing, training, and much more, can have an impact on the relationship (Hausberger et al., 2008; Scopa et al., 2020). It is necessary to filter out the appropriate, more "general" elements that cause disruptions and associated relationship problems. There has been an increase in studies on this topic in the last decade and scientific interest is growing, but the knowledge gained from them remains limited (Luke et al., 2022). Clearly more research is needed in this area.

Horses are naturally flight animals, so stress is a critical factor that can trigger the flight instinct in them, lead to unpredictable behaviour, and affect both their welfare and

overall safety. As mentioned, Keeling et al. (2016) found that miscommunication can lead to such events. Mindfulness in communication is significant in building and maintaining a relationship (Merkies & Franzin, 2021). Communication is used for social interaction and is a crucial factor for maintaining a relationship and can be especially challenging in interspecific relationships (Calvi et al., 2020). Communication occurs either consciously or unconsciously and can be divided into three types: verbal, non-verbal and vocal. Humans are proficient in all 3 types, whereas horses communicate only in non-verbal and vocal ways. Nevertheless, due to frequent human contact, horses are also able to interpret verbal utterances of humans as information and seem to be able to perceive human actions very well (Merkies & Franzin, 2021; Schrimpf et al., 2020) from which it can be concluded that most communication errors in the horse-human relationship originate from humans.

The following review therefore deals with human errors in communication, triggered by unconscious behaviour, which lead to an increase in acute and chronic stress in horses.

Stress is a nonspecific response to environmental (external stressors) or psychological (internal stressors) challenges (Baragli et al., 2014) and can act as either a facilitating factor (eustress) or a limiting factor (distress) (Bartolome & Cockram, 2016). Stressors are stimuli from the environment that are strong enough to elicit an acute, adaptive response from the organism or, if confronted excessively with stressors, can lead to a non-adaptive, chronic response and thus have potentially detrimental effects on the animal's well-being (Budzynska et al., 2023). To investigate stress indicators in horses scientifically, behavioural tests or behavioural measures have been developed and evaluated (Hausberger et al., 2008). However, determining more objective, physiological measurements, such as heart rate variability (HRV), is essential (Scopa et al. 2020). Heart rate and heart rate variability are non-invasive measures and the most used physiological correlates of the autonomic nervous system (ANS) for behavioural assessment (Scopa et al., 2020). In addition, to measure stress physiology, either hair, saliva, or stool samples are analysed for glucocorticoid

metabolite levels (Jolivald et al., 2023). These measurements are important because stress-related behavioural indicators may be absent due to certain types of education and training (Merkies & Franzin, 2021). Particularly in the case of chronic stress, associated stereotypic behaviour, altered metabolism, or suppressed immune function provide information (Popescu & Diugan, 2017). However, physiological stress parameters only reflect arousal or activity levels of an individual, but not perceived valence. Therefore, the object of consideration is usually a combination of physiological measurements related to behavioural parameters. For social animals that cooperate with humans, interspecific communication is very important (Lansade et al., 2021). As mentioned earlier, communication is very complex and multifaceted, so it is first important to filter out which aspects of communication can influence stress. Since the sometimes gentle nature of language can have a calming effect on people, it is reasonable to assume that horses respond in the same way and that it can reduce stress reactions (Lansade et al., 2021). However, stress is more likely to be caused by unconscious action (Brubaker & Udell, 2016), so it is also reasonable to assume that more unconscious aspects of communication, such as human body language or facial expressions, trigger responses in horses and increase acute indicators of stress.

Emotions can directly affect our behaviour and thus interactions (Trosch et al., 2020). If horses are able to interpret these changing signals correctly as well, then positive emotions will result in a decreased occurrence of stress responses and negative emotions, on the other hand, will manifest in an increase in stress levels. Thus, humans would be able to influence the reactions of horses to, for example, unfamiliar situations and associated potential risks.

However, the management of potential hazards is also related to the degree of bonding between humans and horses (Hemsworth et al., 2015). The degree of bonding includes the type, quality, and frequency of human contact. As a result, not only can a strong bond of trust be expected to provide more security, but it can also be expected to have a positive long-term effect on potential stress responses. Whereas a low level

of attachment may represent a significant influence on chronic stress (Ijichi et al., 2018).

The purpose of this systematic review is to provide quantitative answers to test these assumptions.

2 Methodology

For this work, a systematic literature review (SRL) was chosen to provide a more comprehensive look at the elements of the HHI that have already been studied, and to provide greater validity than a single study can have. SRLs aim to present a fair assessment of a research topic by using a trustworthy, rigorous, and verifiable methodology (Kitchenham, 2004). This can provide greater clarity on which aspects are more or less significant in improving equine welfare and mitigating acute and chronic stress that threatens horses' health.

For the current review, the approach of Kitchenham (2004) was used as review process. It includes the following five steps: [1] identifying the research question(s); [2] identifying relevant studies; [3] selecting the studies; [4] preparing the data; [5] synthesizing the results.

2.1 Identifying the research questions

The aim of this review is [1] to filter out which aspects of communication influence short-term stress; [2] to find out to what extent human emotions influence short-term stress indicators in horses; and [3] to clarify whether the degree of bonding affects long-term stress indicators. These general research questions served as guidelines for the present SRL:

1.   What aspects of communication influence acute stress?

2.   Do human emotions influence short-term stress indicators in horses?

3.   Does the degree of bonding affect long-term stress indicators?

The elements of the human-horse relationship are already well covered in other reports (Contreras-Aguilar et al., 2019; Fureix et al., 2009; Hausberger et al., 2008; Henry et al., 2005; Ringhofer & Yamamoto, 2017; Sankey, Henry, et al., 2010). However, based

on current research, this is the first review summarizing the effects of the horse-human relationship on stress and stress-related health problems in domestic horses.

2.2 Identifying relevant studies

For the search process, the electronic databases Web of Science was searched, as it meets the following criteria:

- o Web of Science is accessible through our institution
- o The database has various filtering options, such as the language and time of publication
- o Search strings can be used for a more targeted literature search
- o The database can use logical expressions or a similar mechanism.
- o The database allows full-text searching or searching only in specific sections of works.

From the preliminary search of human-horse interaction studies, text words used in titles and abstracts were examined to identify additional keywords, which were applied in the subsequent, more comprehensive search process.

The following keywords were identified: horse-human relationship; interaction; short-term stress; long-term stress; influence of human behaviour on horses; communication.

Based on this, for a more targeted and comprehensive search, these five search strings were used:

- o (human-horse relationship) OR (human-horse interaction) AND (stress)
- o (the influence of human behaviour on horses) AND (stress)
- o (short-term stress in horses) OR (acute stress in horses)
- o (long-term stress in horses) OR (chronic stress in horses)
- o (human horse communication)

Furthermore, in order to identify relevant studies in accordance with the research questions and to facilitate the search process, inclusion and exclusion criteria were established (Tab. 1). Only articles that specifically focused on human-horse interaction and related stress were included. It was also important that their effects related to domestic horses and not wild horses or other species.

Table 1: Inclusion and Exclusion criteria

Inclusion criteria	Exclusion criteria
Papers reporting on the effects of human interaction on the domestic horse	Scientific studies that do not focus on the HHI or report on the effects on humans
Primary studies, peer-reviewed	Reviews, opinion pieces
	Articles for which the full text is not available
Studies written in English and German	Studies in other languages
Domestic horses (equus callabus), all breeds	Wild horses
Psychosocial factors that trigger stress	other stress-causing factors, such as sport, breeding, training methods, nutrition, husbandry
Short-term stress	
Long term stress	

Studies were excluded that were not available as full text and were neither written in German nor in English, and in order to preserve quantitative research, publications that did not contain primary data. Also excluded were other stress-related factors such as husbandry, nutrition, breeding, sport, or training methods.

2.3 Selection of the studies

The identified articles were carefully selected using the previously determined search strings, a title and abstract screening, followed by a screening of the full text articles. Articles that did not meet the criteria and duplicates were removed.

Articles that were to be included from citations were also subsequently subjected to full text screening and a review of inclusion and exclusion criteria.

2.4 Preparation of the data

The literature results were presented as a concept matrix (Tab. 2) according to Webster and Watson (2002) in order to better capture the characteristics of the collected data and to make them graphically visible.

Table 2: Conceptmatrix, Webster and Watson (2002)

Paper		Concepts											
		Aim		Object of consideration					Parameters of the state detection				
Author	Year	Measurement	Data Analysis	Stress	Behaviour	Communication	Emotions	Bonding	HF	HRV	Cortisol	Behaviour	Interview
Chamove et al.	2002	X			X							x	
Rietmann et al.	2004	X		X					X	X		X	
Brandt	2004		X			X							X
Christensen et al.	2005	X			X				X			X	
Henry et al.	2005	X			X							X	
Birke	2007		X		X	X							X
Ligout et al.	2008	X			X							X	
Hausberger et al.	2008		X		X	X		X					
Birke	2008		X			X		X					X
Fureix et al.	2009	X			X							X	
McGreevy et al.	2009		X		X	X							
Mendl et al.	2009		X		X		X						
Sankey et al.	2010a	X			X							X	
Sankey et al.	2010b	X						X				X	
Krueger et al.	2011	X			X			X				X	
Baragli et al.	2011	X			X	X			X			X	
Gorecka-Bruzda et a	2011	X			X							X	
Lampe and Andre	2012	X			X	X						X	
Savvides	2012		X			X							X
Young et al.	2012	X	X	X	X				X		X	X	
Popescu and Diugan	2013	X						X				X	
Merkies et al.	2014	X		X					X			X	
Baragli et al.	2014	X					X			X		X	
Payne et al.	2015		X	X	X		X	X					
Hemsworth et al.	2015		X					X					
Costa et al.	2015	X			X							X	
Wathan et al.	2016	X				X			X			X	
Malavasi and Huber	2016	X				X						X	
Smith et al.	2016a	X					X		X			X	
Olczak et al.	2016		X	X	X								
Bartolome and Cock	2016		X	X									
Brubaker and Udell	2016		X		X								
Chapman and Thomp	2016		X		X								
DeAraugo et al.	2016		X		X								
Smith et al.	2016b	X				X			X			X	
Villas-Boas et al.	2016	X		X						X	X	X	
Keeling et al.	2016	X			X							X	
Prendergast et al.	2016	X			X	X						X	
Popescu and Diugan	2017	X		X								X	
Hartmann et al.	2017		X		X	X							
von Borstel et al.	2017		X	X					X	X	X		
Ringhofer and Yama	2017	X				X						X	
Ijichi et al.	2018	X		X				X	X	X			
Smith et al.	2018a	X				X						X	
Kozak et al.	2018	X			X		X		X			X	
Smith et al.	2018b	X				X						X	
Nakamura et al.	2018	X					X		X			X	
Proops et al.	2018	X				X	X		X	X		X	
Scopa et al.	2019		X			X	X						
Contreras-Aguilar e	2019	X		X							X		
Henriksson et al.	2019	X			X								X
Baba et al.	2019	X				X	X					X	
Ladewig	2019		X		X	X							
Chapman et al.	2020		X		X								X
Lundberg et al.	2020		X		X								X
Scopa et al.	2020	X						X		X			
Sabiniewicz et al.	2020	X		X		X						X	
Arrazola and Merkie	2020	X	X	X	X			X	X				X
Calvi et al.	2020		X			X	X						
Lesimple	2020		X		X								
Schrimpf et al.	2020		X				X					X	
Trosch et al.	2020	X				X			X			X	
Kelly et al.	2021		X	X				X					
Lerch et al.	2021	X			X							X	
Brubaker et al.	2021	X						X				X	
Merkies and Franzin	2021		X			X						X	
Lansade et al.	2021	X				X						X	
Hemsworth et al.	2021		X		X								X
Anzulewicz et al.	2021		X		X								
Riva et al.	2022	X			X							X	
Lichtmann et al.	2022	X						X				X	
Merkies et al.	2022	X				X			X			X	
Maigrot et al.	2022	X					X					X	
Luke et al.	2022		X	X	X								
Jolivald et al.	2023	X		X							X		X
Budzynska et al.	2023		X	X									

3 Results

3.1 Selection of the included articles

Having used the previously selected search strings, a total of 950 records were identified in the Web of Science search engine, started on 02/16/2023 and completed on 03/15/2023, excluding additional included datasets from Citations. After removal of 117 duplicates, a title and abstract screening followed in which 760 studies were excluded. 388 records were irrelevant equine studies, 269 irrelevant clinical studies, and 107 records were not related to domestic horses. Of the remaining studies, three were unavailable and were excluded. The literature review process (Fig. 1) identified nine relevant sources from citation searching. A total of 76 studies were included in this review.
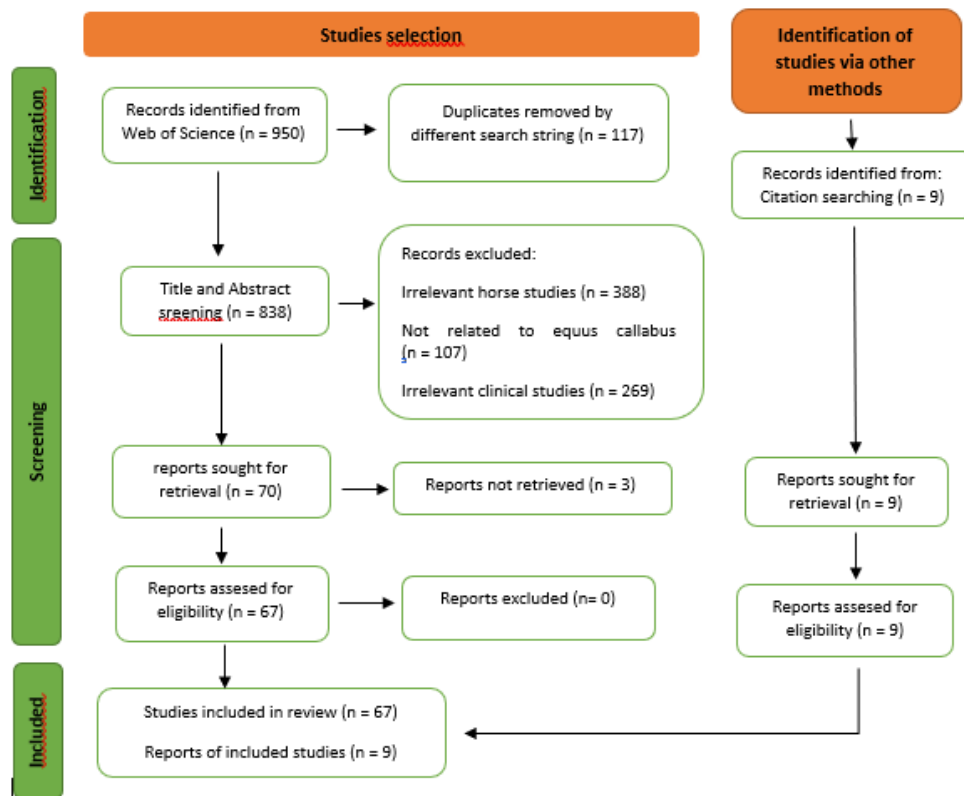
Figure 1: Methods of selection of studies

3.2 Article characteristics

3.2.1 Study populations

From all included studies, a total of 4256 horses were used to investigate the HHI. The size of each study population varied widely, from one horse to 715 horses. Age also varied widely. Overall, the studies span an age range from the first 5 days of life to 29 years of age. Only a portion of the included studies reported the sex of the horses. They indicate that 1,199 mares, 910 geldings, 176 stallions, and 64 foals were used. The missing data came from studies with a very large population size over different locations.

3.2.2 Aspects of Human-Horse Interaction

Of the selected studies, 13 papers dealt with handling, 21 with the non-contact form of handling, and 10 studies with a combination of the two forms. In addition, there were 10 surveys that dealt with the personal opinions of the owners regarding the general handling.

3.2.3 Years of publication

The publication years of the included studies range from 2002 to 2023, of which most were published in 2016 and two-thirds of all papers were published in the same or subsequent years.

3.2.4 Description of the studies

This chart (Fig. 2) on the respective study parameters illustrates that behavioural tests were the most commonly chosen means of studying the HHI and tended to be less supported by physiological measurements. In particular, long-term stress indicators such as glucocorticoid metabolites in hair were rarely examined.
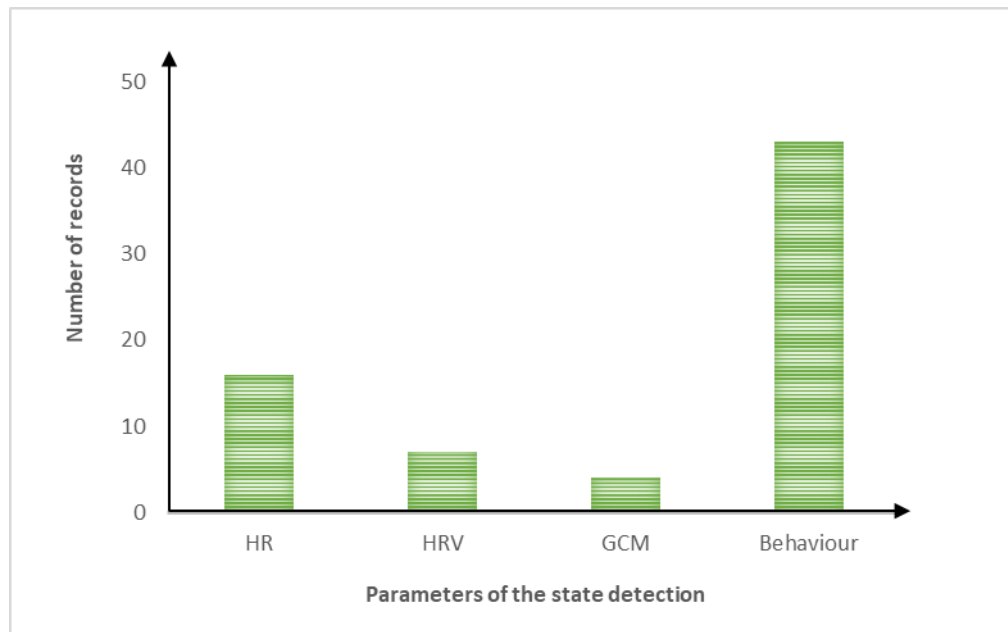
Figure 2: The measured stress parameters in the included articles

A control group was present in only 20 studies.

In studies of emotion discrimination, 6 papers examined the effects with positive and negative conditions, but only one paper included a neutral study condition.

3.3 Measurements of the horse

A total of 8 studies used purely physiological measurements, 28 papers used behavioural observations, and 13 studies used both types of measurements.

3.3.1 Physiological measurements

Sixteen studies examined the effects of HHI using heart rate (HR) using a Polar HR monitor, seven studies determined heart rate variability using a portable electrocardiogram (ECG), and four studies examined the levels of glucocorticoid metabolites (GCMs) in faeces or glucocorticoids (GCs) in blood or in saliva samples

from the horses. Two studies also looked at eye temperature and other salivary measures.

3.3.2 Behavioural measures

Behavioural data were collected via direct observation in 12 articles and via video analysis in 19 articles. The data obtained were assessed in 12 studies using scales described in the literature, and in 14 studies using self-developed behavioural scales.

3.4 Thematic object of observation

As shown in the concept matrix (Tab.2), 30 articles were dedicated to data analysis and 46 studies to measurement and were all listed in the thematic figure (Fig. 3) to give an overall view of the topics. In the evaluation of the studies, the following diagram emerged and shows that almost half of all articles dealt with general behaviour and only a quarter of all articles explicitly referred to stress. Of the three selected subjects, the topic of communication was explored much more frequently than bonding or emotions.
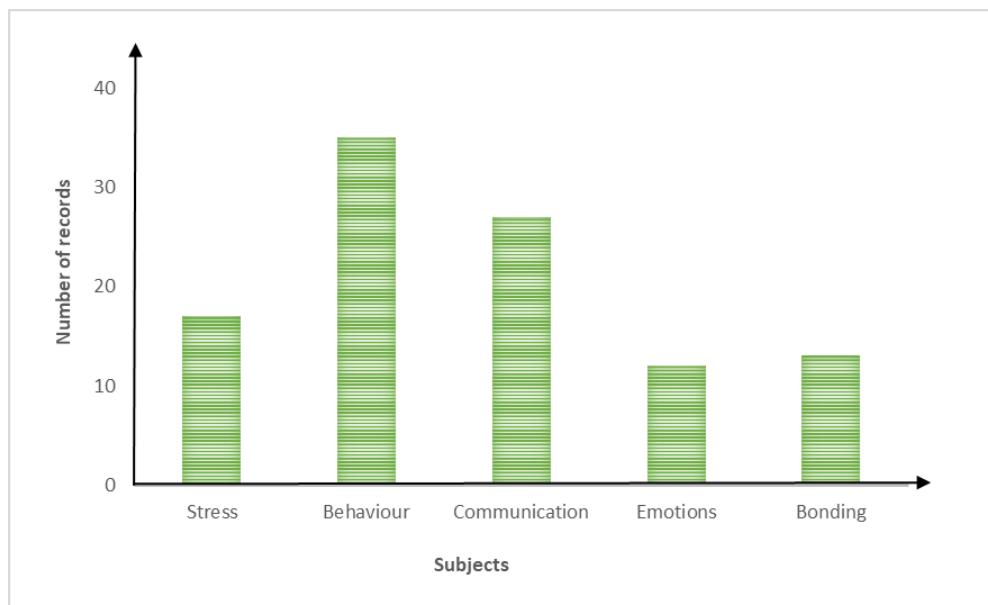

Figure 3: Object of consideration

In order to explore the meanings and impacts of the HHI elements in the best possible way, special attention was paid to the measurements that explicitly addressed the topics of the research questions. Thus, 11 studies on the topic of communication, 7 studies on the topic of emotions and 12 studies on the topic of bonding emerged out of 46 studies with measurements.

Four elements emerged from the measurements on communication: preferences for certain stimuli, attentional state, type of language and odours (Tab. 3).

Table 3: Elements and results of communication

Communication elements	Task	Results	References
Preferences for certain stimuli	Receiving a food reward during the influence of visual, olfactory and auditory stimuli	Increase in HR to visual and auditory stimuli only, but not to olfactory stimuli; shorter feeding time during food reward and increased vigilance.	Christensen et al. (2005)
	Object selection by means of combined and conflicting visual and auditory stimuli	Horses prefer visual stimuli; when horses are influenced by more than one stimulus, they perform worse	Prendergast et al. (2016)
	Visual and olfactory stimuli of a person in comparison with a congruent/incongruent voice	Cross-modal recognition of a familiar person	Lamp & Andre (2012)
	Positive/negative human facial expressions combined with positive/negative human voices	Horses can perceive human emotions cross-modally and recognize them cross-modally by facial expressions and voice	Nakamura et al (2018).

Attention state	Obtaining an unreachable object by sending signals to people	Horses can flexibly use different communication channels to get human attention	Ringhofer & Yamamoto (2017)
	Eye-tracking task in which horses were asked to follow the gaze of a familiar/unfamiliar human to reach three feed buckets at different distances	The horses were more oriented to the attention state of a familiar human than to that of an unfamiliar one	Krueger et al. (2011)
	The horses should communicate their intentions to get a certain object to a human being	Horses adapted their communication channels to gain the attention of humans and are thus capable of referential communication	Malavasi & Huber (2016)
	Effects of HHI in a free environment compared to a constrained environment.	The restricted environment negatively influences behaviors and stress responses	Baragli et al. (2011)
Pet directed speech (PDS)	Use of adult language compared to PDS in grooming and following cues.	On PDS, the horses responded better and showed more relaxed behaviors	Lansade et al, (2021).
Posture	Approach behavior towards a human dominant and submissive body posture.	Horses prefer to approach people with submissive posture	Smith, Wilson, et al. (2018).
Odours	Sniffing human Fear/non-fear odour	Horses can perceive human emotions by chemosignals	Sabiniewicz et al (2020).

The most important elements related to emotions were interspecific communication, transmission pathways, incongruent valence and reactivity (Tab. 4).

Table 4: Elements and results of emotions

Emotion elements	Task	Results	References
Interspecific perception	Picture with human positive/negative facial expression is shown to horses and 3-6 hours later they are confronted with the same person as in the picture	Horses recognize people from photos also in reality and adjust their behavior according to the previously perceived emotions (affiliative/agonistic behavior)	Proops et al. (2018)
	Test subject with positive/negative visual and auditory emotion expressions gives the horse cues about a new object	Human emotional expressions influence the horse's behaviors positively/negatively	Schrimpf et al. (2020)
	Showing a videotape of a positive/negative HHI with a known/unknown individual.	Horses indirectly attribute valence to people through observation (respond to valence in the same positive/negative way)	Trosch et al, (2020)
Transmission paths	Eye tracking and perception in human emotional cues (positive/neutral/negative).	Horses are sensitive to human emotions	Baba et al. (2019)
Incongruent valence	Distinction between inter- and intraspecific vocalizations with opposite valence.	Horses, regardless of species, respond to emotional vocalizations, especially more strongly if the initial stimulus was negative.	Maigrot et al, (2022).
Reactivity	Everyday handling tests and reaction to new objects with horses of different characters	Reactivity is not a property, but is composed of a sum of internal and external factors	Kozak et al. (2018)
	Challenging floor exercise (3 minutes walking backwards)	Duration of stress increases level of arousal; HR and HRV increased as stress increased	Rietmann et al. (2004)

Familiarity and quality emerged as core elements in the theme of relationship (Tab. 5).

Table 5: Elements and results on the type of bonding

Relationship elements	Tasks	Results	References
Familiarity	Handling tests with familiar/unknown persons	Familiarity can reduce stress reactions	Scopa et al. (2020)
	Everyday interactions with food reward for 6-8 months.	Positive interactions lead to positive relationships	Sankey et al. (2010)
	Handling tests, separation and return with a familiar/unknown person.	Horses show bonding related behaviors	Lundberg et al. (2020)
	Handling tests with a familiar/unknown person	Familiarity is less crucial than competence	Ijichi et al (2018).
Quality	Course with leadership of people of different settings	The human attitude influences the behavior of the horse, if this is positive, it reacts with affiliative behaviors	Chamove et al. (2002)
	Effect of Equine Asissted Interactions (EAI) horse work on their perception of humans.	EAI horses exhibit more affiliative behaviors toward insecure and unfamiliar people than toward secure, familiar people	Arrazola & Merkies (2020); Lerch et al. (2021).
	Unsolvable task for horses to get food, must send appropriate signals to humans to achieve their goal	The size (horse or pony) and personality of the horse influence the human-related behaviors	Henriksson et al. (2019)
	Handling tests with approach, forced and non-forced familiar human contact.	Suboptimal human-horse relationships lead to more frequent occurrence of agonistic behaviors	Costa et al. (2015)

	Type of approach, work-related and unfamiliar tasks with a person after several months.	Horses remember previous interactions and their meaning	Fureix et al. (2009)
	Handling tests with foals (forced contact/free contact/no contact), repetition after several months	Foals with forced human contact showed more agonistic behavior initially, but were also more controllable than untreated foals. Nevertheless, the effect of early handling did not last longer than 4 months	Ligout et al. (2008)
	Positive interactions with mares in the presence of foals	The positive mare bonds had a lasting positive effect on the foals, so that they still attributed a positive value to humans 1 year later	Henry et al. (2005)

4 Discussion

4.1 Validity of the stress measurement

Methods to measure GCs or their metabolites need to be carefully validated (Sheriff et al., 2011). Since examining the validity of the data collection is beyond the scope of this paper, the included studies must be critically evaluated.

4.2 Which aspects of communication influence acute stress?

During domestication, both humans and horses have had to find a common level of communication and have developed specific skills to respond to and interpret each other's signals accordingly. A study by Malavasi & Huber (2016) showed that horses use a form of referential communication at both interspecific and intraspecific levels. This means, for example, that through individual experiences with humans, they are able to adapt their communication strategy after failures and flexibly switch their communicative signals. Furthermore, the authors noted that horses are guided by the attentional state of humans to adapt their communication channels accordingly, which is confirmed by results of other studies (Nakamura et al., 2018; Proops et al., 2018; Scopa et al., 2020). Since horses have a wide variety of communication channels (visual, auditory, olfactory, haptic), Proops et al. (2018b) addressed the question of whether horses have certain preferences regarding a wide variety of stimuli. The results showed that horses are very good at using both visual and auditory stimuli and that it is more the strength of each stimulus that matters. However, because the focus of communication in horses, unlike humans, is mainly non-verbal and vocal, a language barrier may still occur. Humans are less aware of the multiplicity of signals they send out (Ladewig, 2019) and their effect, although they make up a large part of interactions and are perceived by horses. Horses are very sensitive to vocal utterances (Baba et al., 2019; Lampe & Andre, 2012; Lansade et al., 2021; Nakamura et al., 2018), body language (Birke, 2007, 2008; Brandt, 2004), smells (Christensen et al., 2005; Sabiniewicz et al., 2020) and can remember facial expressions very well (Proops et

al., 2018; Smith et al., 2016a, 2016b; Wathan et al., 2016). Each individual aspect of communication has an effect when dealing with horses and especially on acute stress, but only once they are linked to emotions and attributed a value (Birke, 2007; Brandt, 2004; Scopa et al., 2019), as explained in more detail under 4.3. A significant and essential part that is crucial for joint communication, however, is proper socialization, as Ladewig (2019) pointed out, because horses develop their intraspecific communication skills through social learning and observation, and otherwise misinterpretations and disruptions in the joint language level occur.

4.3 Do human emotions influence short-term stress indicators in horses?

Emotions are an important component of social life. Especially in flight animals, they provide valuable cues to threats and are an important component to ensure their survival. In addition, they help predict the behaviour of others and respond with situationally appropriate behaviours (Baba et al., 2019). Horses can recognize emotions both inter- and intraspecifically (Maigrot et al., 2022). They respond to negative emotions with agonistic expressions and exhibit stress responses in addition to avoidance behaviours (Baba et al., 2019; Wathan et al., 2016). To improve the human-horse relationship and interactions, it is important to understand how horses respond to emotional cues from humans and use them to adjust their behaviour or react in uncertain situations (Calvi et al., 2020). Horses have a type of categorization process in which they evaluate the nature of human actions as positive, neutral, or negative stimuli (Scopa et al., 2019). When emotions are considered from the perspective that positive as well as negative emotions can activate processes that can range from calming to anxiety, which can be measured physiologically and behaviourally, the major impact on the human-horse relationship becomes apparent (Scopa et al., 2019). It is a matter of effectively managing emotions in tense situations, as particularly stressed horses behave more aggressively towards humans (Rietmann et al., 2004).

Emotions are characterized by two dimensions: valence and arousal. Valence is understood as an intrinsic attraction (positive valence) or aversion (negative valence), arousal as a relevant signal that is perceived, and both end in a resulting behavioural execution (Payne et al., 2015). However, the perceived situation is also dependent on subjective experience (Payne et al., 2015). Emotions are not simple reflexes but rather a cognitive linkage of content, physiological state, and experiential matching (Merkies et al., 2022). Multimodal studies (Nakamura et al., 2018; Smith et al., 2016a; Trosch et al., 2020)) on vocalization and facial expressions emphasized the cognitive link and the importance of valence. Negative events in particular are more easily remembered than positive or neutral ones (Scopa et al., 2020). A study by Fureix et al., (2009) relying on memory valence showed that perception and response to human interactions can have extreme effects, as even a single negatively valenced interaction, can "wipe out" several previously positive ones, leading to the same outcome as multiple negative interactions alone. This study highlighted the great importance of memories and their implications, especially when they are linked to negative experiences. Because of this effect, certain expectancy responses from the other individual may arise, such as a negative expectancy from the rider/handler being reflected in an increase in heart rate (HR) in their horse (Hartmann et al., 2017). Proops et al. (2018) used a study to illustrate how long-lasting the effects of a single interaction can be. For this purpose, 28 horses were shown a photograph with a happy or angry person. Based on perceived valence, the horses responded with either affiliative or agonistic behaviours three to six hours later in a neutral context with the same person as in the photo. Although these horses had no prior direct experience with the person shown in the picture, they behaved differently based on a single facial expression. Consequently, valence may also determine the quality of the HHI (Hausberger et al., 2008).

Several researchers (Birke, 2007, 2008; Chapman et al., 2020) found out that humans have the power to "activate" or "attenuate" a "fear memory" that exists in horses, and thus "direct" horses' responses to new, unfamiliar situations and social isolation and

their associated anxiety (Villas-Boas et al., 2016). As described in 3.1, communication signals are associated with emotions and can be expressed visually, olfactorily, or acoustically (Baba et al., 2019; Nakamura et al., 2018; Proops et al., 2018; Smith et al., 2016b; Trosch et al., 2020). An individual expressing negative emotions may elicit stress and anxiety responses in the horse, thereby jeopardizing the safety of both, whereas positive emotions may have a calming effect (Trosch et al., 2020). The key factor that determines whether a stressor has a negative, neutral, or even positive outcome is whether or not an individual feels in control of the situation (von Borstel et al., 2017). Olczak et al. (2016) emphasized that anxiousness is an extremely crucial aspect in the horse-human relationship, as mutual safety relies on it and caution is especially necessary with stressed horses, as they may have a lower threshold of perception for emotions and subsequent reactions. Schrimpf et al. (2020) showed in their experiment that fear responses of horses are enhanced in a negative condition. Sabiniewicz et al. (2020) found in their study that horses responded differently to human fear and non-fear odours. When sniffing the fear odour, horses were significantly more alert and alarmed than when sniffing the non-fear odour. However, the sequence of perceived stimuli can also have a different effect: when a positive stimulus is followed by a negative one, horses are more likely to be more relaxed than when the stimuli are in the reverse order (Maigrot et al., 2022).

Horses are sensitive to the mental state of humans, as evidenced in studies by Keeling et al. (2016) and von Borstel et al. (2017). Horses recognized the difference between genuine and acted nervousness (differences in HR). However, the horses responded with a lower heart rate in nervous and anxious humans, indicating that they are not physiologically stressed as a result, and the relationships need to be further explored.

Several studies illustrated how horses use human communicative cues from face, voice and posture to interpret their emotional state. Baba et al. (2019) noted that in an experiment with positive, neutral, and negative emotional facial expressions, horses responded to negative facial expressions with stress responses. This study clearly showed that horses exhibit both physiological and behavioural changes when they

perceive negative human emotions. Likewise with human emotionally charged chemosignals (Scopa et al., 2019). Because horses are highly receptive to emotion, Lansade and colleagues (2021) investigated the extent to which horses are receptive to pet directed speech (PDS). Similar to findings from previous studies that horses' HR decreases and they are more relaxed when they hear positive voices (Nakamura et al., 2018; Trosch et al., 2020), horses were generally calmer when spoken to with PDS. However, only mares that had no previous experience with PDS were selected for this study. However, it would be of great importance to determine whether horses that have had negative experiences associated with PDS react differently. Horses do not show any differential responses between female or male human voices, but the valence of the voice has an influence on feeding behaviour in horses (Smith et al., 2018a). Trosch and colleagues (2020) investigated whether domestic horses indirectly attribute valence to humans by eavesdropping on an intraspecific social interaction. Video scenes were presented to them, and behavioural and physiological changes were recorded. The results showed not only a difference in HR, but also that the horses were prompted by the information observed in the video to respond (positively/negatively) with a change in behaviour in real life when confronted with the previously shown subjects. These results provided important evidence that emotional contagion, a transfer of emotions between individuals via some signal, is an important component for social coexistence in herds. Especially sharing positive emotions can strengthen social bonds, which in turn also has an important function for the HHI. However, emotional contagion can also negatively affect future interactions (e.g., loading, farrier, veterinarian) that are perceived as stressful for horses (Trosch et al., 2020).

Mendl et al. (2009) investigated whether horses, like humans, are subject to judgment bias due to perceived emotions, or an underlying emotional state. Their hypothesis, namely that horses with a baseline negative emotional state are more likely to evaluate the cues as a negative event than when they have a baseline positive emotional state, was confirmed and is consistent with the results of Lesimple (2020). Poor health

causes horses to respond more pessimistically to an ambiguous stimulus than horses in good health.

Human attitudes always reveal some "evaluative tendency" and have as much influence on behaviour, as Chamove et al. (2002) described. It should be noted that human attitude may correlate with horse behaviour, but this needs further investigation. Scopa et al. (2020) investigated whether long-term positive human-horse relationships also had an influence on the horse's achievement state. It was not only shown that their assumption has been correct, but also that horses were less able to distinguish between positive and neutral conditions. This is consistent with results of a later study by Merkies et al. (2022) in which ponies moved away from the subject due to positive facial expressions, whereas they responded to negative facial expressions with curiosity expressing behaviours, rather than affiliative behaviours. The authors noted that horses may have a harder time distinguishing between positive and neutral emotions because they pose less of an immediate threat and do not require action. However, prior experience with humans may have played a role in this. The study by Merkies et al. (2022) highlighted the importance of including the behavioural responses of horses in addition to physiological measurements. There is a degree of conflict between a particular event and the animals' expectations, which can alter the animals' emotions and reactivity. In turn, reactivity is determined by fearfulness, curiosity, and motivation (Kozak et al., 2018). Because in the wild, horse survival is dependent on the classification of the perceived stimulus, and horses may react more violently to new, unfamiliar objects or situations, such as flight, horses with less human experience may interpret a common encounter as potential danger and experience even stronger negative emotional states. Therefore, proximity to humans was interpreted as a sign of "positive recall" (Hartmann et al., 2017).

4.4 Does the degree of attachment affect long-term stress indicators?

An interaction results from reciprocal behaviours of individuals, which can be both positive and negative. As seen in 4.2, the valence of any interaction determines its success and forms the core for a good relationship. The particular interaction experiences horses have with people create an important foundation (Fureix et al., 2009), because the relationship builds on it and a bond is formed through mutual familiarity (Hemsworth et al., 2015). The strength and influence of an attachment is especially apparent when the individual is stressed (Bartolome & Cockram, 2016). Lundberg et al. (2020) investigated whether horses exhibit attachment-related behaviours toward humans: Closeness (that is, preference for the attachment figure's proximity in stressful situations), safe haven (relief from stress due to the comfort and support provided by the attachment figure), secure base (increased exploration due to the feeling of security), and separation anxiety (the feeling of distress when the attachment figure is absent). However, only the features of separation anxiety and safe haven could be demonstrated in the behavioural and physiological responses. This may be due to the fact that horses are less dependent on attachment than other domesticated animals, such as dogs (Ijichi et al., 2018). Nevertheless, horses and humans are both capable of establishing, maintaining, and sustaining a relationship with each other based on emotional fine-tuning (Scopa et al., 2019). In particular, behavioural flexibility and cognitive plasticity confirm the relational capacity of horses (Scopa et al., 2019). They can remember individuals for a very long time (Brubaker & Udell, 2016) and recognize them by matching a wide variety of stimuli (Scopa et al., 2019).

Scopa et al. (2020) studied whether long-term positive horse-human relationships had a positive emotional impact on the horse. In their study, horses showed distinguishable emotional responses. A few horses responded with significantly more relaxed behavioural responses when groomed by their trusted human, while other horses did not. The authors concluded that the influence, besides the familiarity to the horse

handler, is rather dependent on the type of interaction. "Grooming" (crawling at the withers) leads to a decrease in heart rate, whether it is performed by humans or conspecifics (Scopa et al., 2020). However, a decrease in heart rate is not guaranteed evidence of whether horses perceive the interaction as positive and promotes bonding (Sankey et al., 2010). However, both humans and horses like to seek physical contact (Ringhofer & Yamamoto, 2017), so this is readily considered to promote bonding. In a study by Krueger et al. (2011) horses did not show any differences in behaviour towards unfamiliar and familiar humans, because - when they want to get food, as for example in this study - they tended to orient themselves to the attentional state of the human (Malavasi & Huber, 2016; Ringhofer & Yamamoto, 2017) as opposed to the human's identity (Krueger et al., 2011). This is in contrast to the findings of Smith et al. (2016). Using human emotional facial expressions, it was found that horses' responses were stronger with unfamiliar humans than with familiar ones. The authors suggested that because of familiarity, there may be some flexibility and compliance, including matching previous experiences with that human, whereas unfamiliar stimuli always elicit increased alertness in horses, as confirmed by the results of Nakamura et al. (2018) in their study of interactions of emotional congruence and familiarity. However, appropriate use can also lead to a change in behaviour toward unfamiliar people, as shown in a study of therapy horses and their significant preference for unfamiliar people (Brubaker et al., 2021).

Familiarity can be a stress buffer (Krueger et al., 2011; Payne et al., 2015), but building trust and a strong bond takes time. This is often not given when horses change owners frequently, as the results of Liehrmann et al. (2022) and others (Hartmann et al., 2017; Ijichi et al., 2018) showed. A lack of, or even suboptimal, relationship between humans and horses can lead to both longer approach times and more frequent avoidance behaviour (Riva et al., 2022). Differences in relationships result from the behaviour of the human, to an attitude toward horses, and the experience of the horse (McGreevy et al., 2009). However, rather than minimizing stress responses in horses, as mentioned earlier, the mere presence of a human can cause stress in a horse

(Gorecka-Bruzda et al., 2011), for example, when a horse is constantly overworked in training (Anzulewicz et al., 2021) or contact is forced (Ligout et al., 2008). A relationship can range from trust to fear and implies different emotions depending on the perceived importance of the interaction (Costa et al., 2015).

However, in addition to quality, the frequency of HHI is also important (Hemsworth et al., 2015). Payne et al. (2015) showed, in a study in day-to-day interactions with different caregivers, the importance of regular interactions and that they have a corresponding effect (positive or negative) on unfamiliar people. Establishments with experienced caregivers provided more positive behavioural response in general concerning interactions with unfamiliar people. An interplay of positive experiences, trust, and human competence makes horses more relaxed, calm, interested, and compliant (Chamove et al., 2002; Ijichi et al., 2018).

In interactions and for bond-building, habituation and desensitization minimize the fear level and stress reactions of horses (Budzynska et al., 2023). This is especially the case when dealing with foals. In this regard, a study by Henry et al. (2005) already drew attention to the fact that longer-term success in handling growing horses results from the use of mare bonding. That is, positive interactions with mares shape the foal better than common practices, such as forced human contact to handle, as described in Ligout et al. (2008). Lack of knowledge can lead to impairments and disturbances in the HHI (Ringhofer & Yamamoto, 2017) and even promote stress reactions. However, to what extent, especially as far as long-lasting stress reactions are concerned, remains to be explored.

4.5 General discussion

From the results, it appears that the type of stimulus is less important than the overall context, the environment of the challenges (Baragli et al., 2014). Thus, it is less a matter of exploring which senses are the most important (Lampe & Andre, 2012) but rather which combinations of stimuli lead to which results (Sabiniewicz et al., 2020).

Since horses are prey animals by nature, any response is founded on an interplay of many stimuli influenced by the environment. Fundamentally, the question is whether humans are viewed by horses more as predators or conspecifics. McGreevy et al. (2009) suggested that from the ground, the horse views humans as conspecifics, but once on their backs they no longer do and it is a matter of teaching unusual, predator-like behaviours to the horse in such a way that they do not cause discomfort, they become accustomed to them and create positive associations. This can be used to shape the horse's attitudes and responses and integrate an important aspect in the HHI, considering that horses can remember an individual for up to 10 years due to their long-term memory (Brubaker & Udell, 2016; Scopa et al., 2019). All human actions, handling, and training influence the memory that is formed, and these create expectations (Fureix et al., 2009). In their study, Nakamura et al. (2018) showed what happens when expectations are not met. Horses respond with more vigilance and are more mindful. It follows that when humans behave differently all the time, it is more difficult for horses to remain calm (Trosch et al., 2020). On the one hand, in connection with negative emotions, this can create a cycle through stress, up to accidents. On the other hand, a good combination of positive touch effects, combined with emotional transference, can form the backbone of a relationship and improve not only further joint interactions but also the horses' resilience and social behaviour (Scopa et al., 2019, 2020).

In practice, however, it is apparent that equine interactions are often viewed with an overly human perspective: however, because humans are sensitive to tactile stimuli, they often assume that petting or other forms of petting animals will have positive effects (Sankey, 2010). Humans, because of their anthropocentric view, usually send unclear signals and think the horse knows what is wanted of it (McGreevy et al., 2009) and many interactions do not match the horses' needs (Hartmann et al., 2017). It is important to pay attention to the horse's psychology, as such unclear signals can cause stress reactions (Bartolome & Cockram, 2016). Equine psychology should be taken

into account to avoid future hazards, and also to keep in mind that different areas of activity require different skills (DeAraugo et al., 2016).

Understanding the forms of interaction between human and horse form the most important basis for drawing conclusions about possible training methods, as described for example by Birke (2007 and 2008) in her articles on Natural Horsemanship. With the right kind of communication, it is possible to reduce the horse's association with the human's actions as predatory responses, and confusion and fear are reduced. With an increased level of excitability and anxiety, they are significantly less motivated to work with humans (Calvi et al., 2020). In general, "role thinking" (conspecific or predator) is not particularly helpful, as humans communicate in a more diverse (e.g. verbal communication) and different way than horses, and have different abilities. Likewise, dominance thinking and acting is not always beneficial because even in herds, roles are dynamically distributed (Hartmann et al., 2017). The anthropocentric view can be a big problem, as it can lead to judgment bias, less mindfulness, and misinterpretation of signals, and most often ends in suppression of horses' natural behaviours (Hartmann et al., 2017). Learning on both sides is important to better assess and understand each other (Anzulewicz et al., 2021). The HHI is not a rigid but dynamic system of reaction and counter-reaction that requires awareness of both actions. Even though people want to be in control, it is also necessary to have a control over oneself, one's own actions and a willingness for personal change/development. Many owners are unable to assess both their own behaviour and that of the horse, which leads to conflict, and the human usually tends to overreact and sometimes use violence in such situations (Birke, 2007; Savvides, 2012).

The study by Savvides (2012) drew attention to the potential for conflict in HHI and emphasized that much more research should focus on these conflicts and their solutions, especially since conflict often occurs in practice. According to Savvides, conflict is characterized by lack of understanding that results from the absence of language. A conflict puts into question the previous relationship, and with the resulting lack of communication, a new space for communication in partnership is created at the

same time. The author suggested that it is important to find a way together to sustainably improve the well-being of both human and horse (Savvides, 2012). According to Savvides, conflicts can be seen as a fresh chance to begin again. The first step is to be aware of the fact that the relationship is a dynamic system. It is a challenge based on complex interrelationships and it should not be approached in isolation, because doing so only leads to short-term corrections and generally bad results (Luke et al., 2022). If all circumstances are taken into account, the next step is to observe, in order to anticipate the potential for conflict and intervene accordingly. It is very unlikely to be able to avoid negative HHI altogether, but competent handling can improve a lot, and provide a secure base - behaviour that can impact unknowns. In general, the goal is to improve competence and provide some type of training for horse owners to increase their awareness of the impact of their behaviour on equine welfare (Hemsworth et al., 2021). There is a lack of an unified approach to horse handling, both for common understanding and promoting safety (Chapman & Thompson, 2016). Integration of knowledge, experience, the horse's natural behavioural repertoire, and current practices are essential for an all-encompassing understanding (Brubaker et al., 2021; Hausberger et al., 2008; Merkies et al., 2022; Schrimpf et al., 2020).

Incorrect behaviours on the side of the human, as well as lack of trust, are triggers for fear and stress (Brubaker & Udell, 2016; von Borstel et al., 2017). Fear and unpredictability of the horse are the most common factor associated with accidents (Merkies et al., 2014). Apart from a lack of knowledge, there still remains a lack of certainty about how to remain safe. This is a matter of people's overconfidence and willingness to accept risk. More confident people are more likely to expose themselves to greater risks. For example, many people tend to choose short-cut solutions based on inaccurate, uncertain, or biased decisions, such as preferring to put on a helmet when riding rather than investing in sound training (Chapman et al., 2020).

4.5.1 Future directions

As mentioned earlier, to improve the HHI people must also be willing to work on themselves and find common ground with horse companions. Developing a relationship takes time, time that many people do not have or do not want to take. In future research, it would be beneficial to find out how long it actually takes to develop a relationship and bond. Forced contact can be negative and lead to stress reactions (Ligout et al., 2008). Horses without proper socialization may be more likely to view humans as a threat, thus increasing danger (Baragli et al., 2014; Budzynska et al., 2023). In addition, horses may have different needs and adaptive abilities depending on their experience and handling. To this end, Young et al. (2012) developed a behavioural scale for stress responses in horses, which is considered as a simple and reliable method to identify or indicate stress levels. Thus, horse owners and behaviourists can make use of this scale, which is based on behavioural and physiological measurement in regular routine husbandry procedures. The scale can be supported by the so-called Broken/Unbroken Test (BUT), to determine behaviour in horses with different sensitivities (Riva et al., 2022). Finally, more research is needed to determine whether horses perceive interactions as rewarding or stressful (Ligout et al., 2008).

5 Conclusion

The purpose of this review is to determine whether human errors in HHI, triggered by unconscious behaviour, lead to an increase in acute and chronic stress in the domestic horse. This assumption could be confirmed to some extent, as a number of studies have shown that deficits in handling practices exist, which can lead to relationship problems between humans and horses. These are expressed as acute behavioural as well as physiological stress responses.

In order to find a common level of communication, it is important that humans develop a sensitivity not only to the horse's communication, but also to themselves, in order to be able to read and understand the signals sent, especially when linked to emotions. Human-horse communication is also a way of learning to deal with one's own emotions. The behaviour, attitudes, experiences, and expectations all have an impact on common interactions. Attention, awareness, correct interpretation of behaviour, consistency in signaling, general consistency and trust are the key elements for a sustainable positive human-horse interaction and relationship. When humans engage in this process, the knowledge gained can make daily interactions much easier and stress reactions can be avoided. It is necessary to create more awareness of these important core elements and also to focus more on emerging conflicts and their solutions. In general, the HHI is difficult to study because of its many influencing factors but the impact of interactions on chronic stress in the domestic horse remains unclear. Future research should more clearly investigate potential long-term consequences.

6 Abstract

Horses are naturally flight animals, so stress is a critical factor that can trigger their flight instinct, lead to unpredictable behaviour and affect both their welfare and overall safety. There is growing evidence that many equine accidents and health problems are due to misunderstandings between humans and horses. This scientific work therefore addresses the interaction between humans and horses and the human influence on acute and chronic stress in the domestic horse. With this systematic review, the previously studied elements of human-horse interaction were assessed to provide more clarity about their meanings and implications. The results showed that any human form of communication, whether verbal, nonverbal, vocal, or via chemical cues, influences interactions but only when combined with emotion has an impact on acute or chronic stress. Horses have a type of categorization process in which they evaluate the nature of human actions as positive, neutral, or negative stimuli and match them to the associated content and subjective experiences via cognitive linkage. This can form an expectation for future interactions and have a lasting positive or negative impact on them, the same way it happens to humans. The results showed that the quality and frequency of interactions between the two individuals have more influence than the degree of familiarity and can be reflected in acute stress responses. The lasting effects of human-horse interactions, especially on chronic stress, are as yet unclear and should be further explored. The systematic review highlights the importance of humans being aware of their communication with horses and its effects in order to maintain a positive, consistent relationship, create safety, and improve horse welfare.

7 Zusammenfassung

Pferde sind von Natur aus Fluchttiere, daher ist Stress ein kritischer Faktor, der ihren Fluchtinstinkt auslösen, zu unberechenbarem Verhalten führen und sowohl ihr Wohlergehen als auch ihre allgemeine Sicherheit beeinträchtigen kann. Es gibt immer mehr Belege dafür, dass viele Unfälle und Gesundheitsprobleme bei Pferden auf Missverständnisse zwischen Mensch und Pferd zurückzuführen sind. Diese wissenschaftliche Arbeit befasst sich daher mit der Interaktion zwischen Mensch und Pferd und dem menschlichen Einfluss auf akuten und chronischen Stress beim Hauspferd. Mit diesem systematischen Review wurden die bisher untersuchten Elemente der Mensch-Pferd Interaktion bewertet, um mehr Klarheit über ihre Bedeutungen und Auswirkungen zu schaffen. Die Ergebnisse zeigen, dass jede menschliche Form der Kommunikation, ob verbal, nonverbal, stimmlich oder über chemische Signale, die Interaktionen beeinflusst, aber erst in Kombination mit Emotionen einen Einfluss auf akuten oder chronischen Stress hat. Pferde haben eine Art Kategorisierungsprozess, bei dem sie die Art der menschlichen Aktionen als positive, neutrale oder negative Stimuli bewerten und über die kognitive Verknüpfung mit dem zugehörigen Inhalt und subjektiven Erfahrungen abgleichen. Daraus kann sich eine Erwartungshaltung für künftige Interaktionen bilden und diese nachhaltig positiv oder negativ beeinträchtigen, so wie es auch bei Menschen der Fall ist. Die Ergebnisse zeigen, dass die Qualität und Häufigkeit der Interaktionen zwischen den beiden Individuen mehr Einfluss hat als der Grad der Vertrautheit und sich in akuten Stressreaktionen widerspiegeln kann. Die nachhaltige Wirkung von Mensch-Pferd Interaktionen, insbesondere auf chronischen Stress ist bislang noch unklar und sollte noch genauer erforscht werden. Die systematische Übersichtsarbeit macht deutlich wie wichtig es ist, dass sich der Mensch seiner Kommunikation mit dem Pferd und deren Auswirkungen bewusst ist, um eine positive, konsistente Beziehung aufrechtzuerhalten, Sicherheit zu schaffen und das Wohlergehen der Pferde zu verbessern.

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9 List of figures and tables

10 List of abbreviations

HHI	Human-Horse Interaction
HR	Heart rate
HRV	Heart rate variability
GCMs	Glucocorticoid metabolites
GCs	Glucocorticoids
ANS	Autonomic nervous system
EAI	Equine Assisted Interactions
PDS	Pet directed speech