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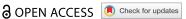
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RESEARCH ARTICLE



Horses' Cardiovascular and Glucocorticoid Responses to Equine-Assisted Therapy with Women with Intellectual Disability: An **Exploratory Study**

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ABSTRACT

Research on equine-assisted therapy (EAT) has primarily been centered on human health. Relatively few studies have addressed the impact of EAT on horses. This study sought to monitor four experienced therapy horses' cardiovascular and glucocorticoid activity over the course of standardized EAT sessions designed to support women with intellectual disability. In the control condition, horses completed the EAT protocol solely with the therapist, thereby resembling a training session. Descriptive data analysis revealed higher levels of heart rate during an experimental EAT session and increased salivary cortisol when horses were navigated by the client through an obstacle course during the "challenge" phase of the protocol, pointing at a greater physical demand due to the recipient on horseback. Given the parasympathetic activity and overall heart rate variability across experimental EAT sessions and the cortisol recovery after the sessions, the findings do not give rise to any acute animal welfare concerns. For a more holistic interpretation of the present research results, further investigation into the horse perception of EAT, based on a bigger sample size and additional markers of welfare, is needed.

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KEYWORDS

Equine-assisted therapy; horse; welfare; heart rate variability; cortisol

Introduction

Accounting for the increasing numbers of animals serving in animal-assisted therapy (AAT) to positively affect human health outcomes (VanFleet et al., 2015), several studies have claimed that the animal perspective should be addressed similarly to the human perception (De Santis et al., 2017; Glenk, 2017). In line with the "One Health" framework, safeguarding animal welfare in AAT is equally important as the focus on human wellbeing (Hediger et al., 2019). Equine-assisted therapy (EAT) refers to human health-directed approaches which are facilitated by interaction with horses. Previous research has shown that client or patient health status, individual attachment style and prior experience with horses influence therapy horses' physiological and behavioral responses to EAT (Arrazola & Merkies, 2020; Kaiser et al., 2006; Mendonça et al., 2019; Merkies

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et al., 2018). Cortisol concentrations were found to be lower in horses when they were ridden by individuals with a disability compared to healthy controls (Fazio et al., 2013). These findings were recently paralleled by a study, in which the effects of being ridden during a hippotherapy session by adult individuals with a diagnosed disability compared to being ridden in a class for adult beginner riders on horses' physiological indicators and behavior was monitored (Potier & Louzier, 2023). More stress-related behaviors emerged in horses under a control condition with little or no human interaction compared to when interacting with veterans with post-traumatic stress disorder (PTSD) (Raman et al., 2023). In another setting, increased physiological and behavioral indices of arousal were recorded during the anticipatory and on horseback phase of EAT in both recipients and horses (Ayala et al., 2021). Other studies did not find any differences in horses' physiological responses (Drinkhouse et al., 2012; Malinowski et al., 2018) or behavior (McKinney et al., 2015) with regard to participation in EAT.

However, a limitation in previous studies using physiological measurements in equines that serve in EAT is the lack of standardization in the protocol that hinders the comparability between sessions. Accordingly, is has been difficult to attribute certain practices in EAT or recipient characteristics to changes in physiological indicators of welfare. To address this gap in the literature, research is challenged to schedule ideal time points for sample collection, incorporate suitable and replicable human-animal interaction sequences into the protocol and apply high standards in data analysis (De Santis et al., 2017).

Physiological responses to stress in horses

In mammals including equines, arousal-related changes in body functions are primarily modulated by activation of autonomic and neuroendocrine cascades, via the sympathetic-adrenal-medullary (SAM) axis and the hypothalamic-pituitary-adrenal (HPA) axis, respectively (Glenk & Kothgassner, 2017). Measurements of the SAM axis response through noninvasive assessment of cardiac activity have been deemed suitable to identify the level of excitement of an animal (Rietmann et al., 2004). The interval between two subsequent heart beats (RR interval) can be analyzed regarding intensity and time. Variation in RR intervals is also referred to as heart rate variability (HRV) that results from deceleration and acceleration of the heartbeat. The standard deviation of all RR intervals (STDRR) reflects the overall variability of the time between heart beats. A higher STDRR indicates a better interplay of sympathetic and parasympathetic branches of the autonomic nervous system. The intensity of parasympathetic activity correlates with the root mean square of successive differences (RMSSD) of adjacent RR intervals (Lohninger, 2017). In frequency domain analysis, high frequency (HF) is merely influenced by the nervus vagus and respiration whereas low frequency (LF) is influenced by parasympathetic as well as sympathetic activity (Baumert et al., as cited in Lohninger, 2017). The ratio of LF and HF (LF/HF) serves as an indicator of sympathovagal balance (Eckberg, as cited in Lohninger, 2017). In non-linear analysis, single RR intervals are plotted against the respective subsequent RR interval. The standard deviation of the diameter of the scatterplot (SD1) examines the variability over single beats in the short term, thereby reflecting parasympathetic activity (Mäkikallio et al., 2004). Stress-related arousal causes hypothalamic neurons to trigger a neuroendocrine cascade which ultimately results in secretion of the glucocorticoid hormone cortisol. Thus, although adaptive in dealing with short-term challenges, high levels of autonomic and neuroendocrine arousal for extended periods of time negatively impact health and wellbeing (Glenk & Kothgassner, 2017).

The main aim of this exploratory study was to monitor the physiological stress response of therapy horses over the course of an EAT session. An acute stress reaction of horses due to EAT would be paralleled in increased heart rate (HR), sympathetic reactivity (LF/HF) and decreased parameters of HRV (STDRR, SD1, RMSSD) in combination with increased cortisol concentrations during and after EAT. Moreover, we sought to address the lack of standardization in previous EAT research using a standardized protocol for EAT and control sessions.



Materials and methods

Study design and procedure

Data collection took place in the riding hall at Lichtblickhof, an equine-assisted therapy center in Vienna, Austria, from February 12 to 25 in 2018. Lichtblickhof offers Equotherapy, a practice of EAT with a focus on non-verbal communication, in which therapy horses are specifically trained to read human body language and facial expressions and feedback those non-verbal impulses to a therapist (Zink, 2008; Zink & Deimel, 2019; Naber et al., 2023).

Therapy horses

Four therapy horses participated in the study: Two mares and two geldings, all four of the breed criollo with an average age of 14.8 years (SD = 5.6) and an average of 9.4 years (SD = 6.6) of experience in EAT (see Table 1). Therapy horses were selected according to their experience in EAT, their familiarity with the therapist as well as their body size to be matched with the clients. Ten female clients, aged between 16 and 27 years (Mn = 21.8, SD = 3.39) and an average of 12.8 years (SD = 4.71) experience in EAT at Lichtblickhof, participated as EAT recipients. All clients had a doctor-diagnosed intellectual disability (down syndrome: N = 4, autism spectrum disorder: N = 2, unspecified neurodevelopmental disorder: N=4) and were attributed a mild (N=5) to moderate (N=5) intellectual disability according to the Glasgow Level of Ability and Development Scale (Cooray et al., 2015). All clients were familiar with the stable environment and knew the horses either from sight or previous interaction.

All horses were healthy and subject to regular (>2 times/year) veterinary checks. Screening for diseases related to altered cortisol metabolism and/or autonomic activity was carried out prior to enrollment in the study. None of the horses took any medications or showed any abnormal behavior. The horses lived in a herd of 20 therapy horses in a spacious open stable which is shown in Figure 1. The area consists of about 570 m² indoor sleeping areas, about 20.000 m² open stable paddock including big sandy area as well as trees and fences to retreat, separated areas and optional enrichment (e.g., bars offering various minerals, self-service brushes at different heights and cut branches to nibble on). Several feeding areas with automated hay racks offering hay as required and water are evenly distributed. An additional pasture area with large meadows and trees is also optionally available for the horses to move, eat, and spend time together. Their everyday routine consists of a balanced mix of EAT sessions, therapy horse training with the therapists (e.g., muscle training, gymnastic exercise, and supervision) as well as break intervals and leisure activities to restore inner balance. On data collection days, the horses were kept in their usual management and housing routine but no additional EAT sessions were performed.

Study protocol

As depicted in Table 1, each horse provided two experimental sessions with the same client and participated in the study with either two or three clients. In total, each horse was involved in four or six EAT sessions, respectively. Each horse participated in one control session with the same protocol

Table 1. Characteristics of participating horses included sex as well as age and experience in EAT (expressed in years). The number of clients and experimental sessions in this study is shown.

Horse	Sex	Age	Breed	EAT experience	Clients	EAT sessions
1	Mare	9	criollo	2.5	3	6
2	Gelding	11	criollo	5	3	6
3	Mare	20	criollo	15	2	4
4	Gelding	19	criollo	15	2	4

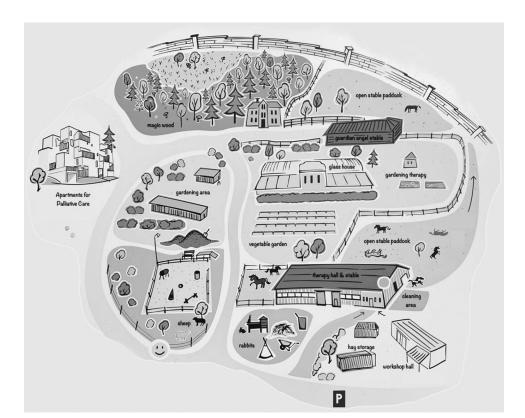


Figure 1. Open stable arrangement.

but without a client (C), thereby solely interacting with the therapist. The study protocol was designed to monitor standardized EAT sessions and included five phases: *Pre-Intervention Baseline*, *Relaxation 1*, *Challenge*, *Relaxation 2*, and *Post-Intervention Baseline* (see Figure 2).

All sessions were guided by a female psychotherapist (37 years old) with longstanding experience (21 years) in EAT and were scheduled within 1:30 to 5:30 pm. Thirty minutes prior to each session, horses were cleaned and prepared for the HR measurements by the same person in a single box in the enclosure "therapy hall & stable" (see Figure 1), which was situated next to the open stable so that visual, acoustic, olfactory or physical contact with the herd was possible at any time. On top of the electrodes horses were saddled with a pad and voulting girth. Halters were used to which a lead string or reins (prior to mounting) were attached – snaffle bits were never used. Horses remained in the single box until the onset of the session. Saliva sample collection was carried out by a previously trained person and was tolerated by all horses without any restraint. At the beginning of each



Figure 2. Study procedure of the standardized EAT sessions. E=Experimental condition, C=Control condition; S1 – S4=saliva samples; __/_ indicates 5 min intervals of HR recording.

session, the first saliva sample was collected (S1). Afterwards, the belt for the heart rate measurements was started. Then, the client and therapist sat down on a chair, with the therapy horse standing next to them without interaction (Pre-Intervention Baseline). Next, the client mounted the therapy horse and therapist lead client and therapy horse two rounds in walking pace through the riding arena. After that, for the guided relaxation exercises, the therapy horse stood calm at a place in the middle of the riding arena (Relaxation 1; Relaxation 2), with the therapist remaining next to the horse and the client on horseback. Between relaxation exercises, clients faced a simple cognitivemotor task moving the horse independently through an obstacle course (Challenge). As depicted in Figure 3, the obstacle course consisted of navigating through a slalom of pylons and construction blocks, crossing over a pole and balancing on a pedestal. During the challenge in the control condition without client, the therapist demanded tasks from the horse being lead through the obstacle course. The therapist announced the tasks from a distance and interfered as few as possible in the interaction between the client and the horse. After the challenge phase, the next saliva sample was collected (S2). Afterwards, the therapy horse was dismounted and the session ended while participating humans sat again on the chair, the horse next to the humans (Post-Intervention Baseline) and a saliva sample (S3) was gathered. All measuring devices were removed and after the horse rested for 30 minutes back in the open stable enclosure and reunited with the herd, a final

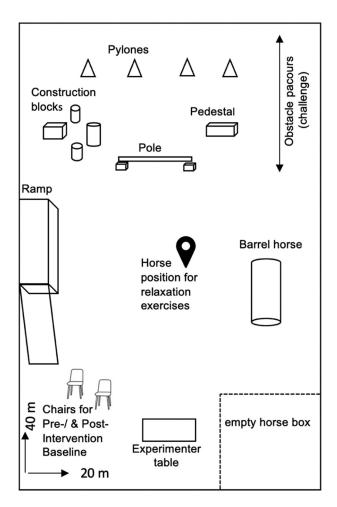


Figure 3. Structure of the riding arena and organization chart for the study protocol.

saliva sample was collected (S4). During the time span between S3 and S4, horses were able to freely engage with the herd.

All EAT sessions took place in the therapy hall (see Figure 1), which includes a closed riding arena. The environment in the riding arena remained unchanged over the entire duration of the study to ensure that the measurements were carried out as standardized as possible. In the riding arena $(20 \text{ m} \times 40 \text{ m})$, there was a ramp for mounting and dismounting the horse, two chairs, the barrel horse, a table on which the technology was set up, and the obstacle course (see Figure 3). The place for the relaxation phases, either on the real or barrel horse, was located in the middle of the hall and remained the same for all measurements.

Operationalization and instruments

Cardiovascular activity was recorded with a Polar V800 telemetric system, with an equine H7 HR sensor electrode base set. For analysis, the sequences of the therapy session were divided into sections of five minutes and analyzed with the program *Kubios HRV* (Tarvainen et al., 2014).

As demonstrated in Figure 1, four saliva samples (pre-during-post EAT exposure) were collected under both conditions, using commercial cotton swabs (Salivette*, Sarstedt, Wiener Neudorf, Austria) that were put into the cheek pouch until saturated with saliva (approximately 60–80 seconds). Afterwards, the swabs were replaced into the device container and immediately frozen at -20° C. Prior to analysis, samples were carefully defrosted on ice and centrifuged at room temperature for 15 minutes at $3000 \times g$. Cortisol in saliva was determined with a highly sensitive enzyme immunoassay (Palme & Möstl, 1997) and $10 \,\mu$ l of a clear saliva dilution (1 + 9 with assay buffer) was used. All samples were assayed in duplicates and average intra- and inter-assay coefficients of variance were less than 10% and 15%, respectively.

Statistics

All data were performed in *Microsoft*Excel*2016 MSO* and analyzed in *IBM SPSS Statistics 23*. Data were analyzed using descriptive statistics and are presented as mean values with standard deviation. While each horse served as its own control, EAT-related values were averaged, depending on whether the horses provided four or six experimental sessions. Intensity of HR, STDRR, RMSSD, LF/HF and SD1 was compared over the course of an EAT session, considering all five phases. Furthermore, the cardiovascular data of each phase was compared to the respective control condition. The median cortisol concentrations (*S1*, *S2*, *S3*, *S4*) were compared according to the study protocol including measurements prior to EAT and after a period of 30 minutes rest post session. Again, the EAT condition was compared to the control condition.

Ethical considerations

All methods and procedures used in these studies were discussed and approved by the institutional ethics and animal welfare committee in accordance with GSP guidelines and national legislation (reference number: ETK-05/01/2018).

Results

According the pilot nature of the study and the small sample of horses, only descriptive data analyses are presented. Any observed increase or decrease in physiological variables can therefore not be attributed to any statistical significance.

As depicted in Figure 4, the HR at the end of EAT (post-intervention baseline) slightly differed from the measurement in the beginning of EAT (Pre-Intervention Baseline). The highest HR was found during the Challenge under both conditions. In both periods of relaxation (Relaxation 1 and

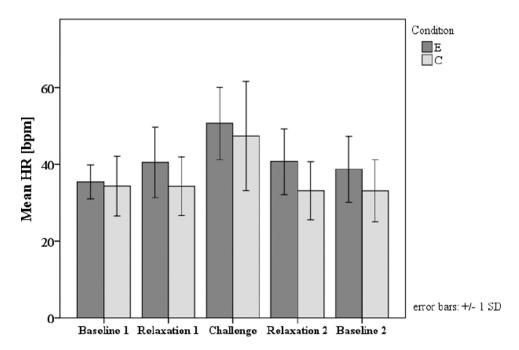


Figure 4. Mean HR (\pm SD) of horses (N=4) compared between the experimental condition and the control condition over the course of a standardized EAT comparing the experimental condition (E) to the control condition (C).

Relaxation 2) for the EAT recipient, the horse HR was elevated in comparison to *Pre-Intervention Baseline* values. Under the control condition, the HR remained on a low level over the course of measurement, except during the *Challenge*.

All HRV results are presented in Table 2. STDRR decreased at the end of EAT (Post-Intervention Baseline) compared to the measurement in the beginning (Pre-Intervention Baseline). It peaked during the Challenge and appeared higher during Relaxation 2 compared to Relaxation 1. The control condition revealed a higher STDRR throughout the study protocol and according to the standard deviation, a higher variability between individual horses (except during the Pre-Intervention Baseline phase) of the protocol.

The intensity of the parasympathetic nervous system activity was higher in the beginning of EAT (RMSSD of *Pre-Intervention Baseline*) compared to the end (RMSSD of *Post-Intervention Baseline*). The RMSSD was highest during the *Challenge* but remained similarly high during

Table 2. HRV (STDRR, RMSSD, LF/HF, SD1) in therapy horses (N = 4) comparing the experimental condition (E) to the control condition (C). Values are expressed as $\overline{X} \pm SD$. Each activity phase (pre-intervention baseline, relaxation 1, challenge, relaxation 2, post-intervention baseline) over the course of a standardized session lasted five minutes.

	Pre-Intervention Baseline	Relaxation 1	Challenge	Relaxation 2	Post-Intervention Baseline
STDRR [ms]	116.2 ± 70.1	92.8 ± 39.1	144.4 ± 68.2	117.3 ± 64.8	83.0 ± 48.5
E C	152.6 ± 75.9	184.1 ± 170.2	199.8 ± 152.0	157.7 ± 214.2	232.0 ± 257.4
RMSSD [ms]	149.2 ± 100.8	108.5 ± 51.6	162.1 ± 84.6	157.6 ± 90.4	105.1 ± 66.2
E C	164.3 ± 97.2	203.1 ± 207.2	196.1 ± 181.4	235.8 ± 346.8	316.8 ± 396.8
LF/HF	3.1 ± 2.3	1.7 ± 0.5	3.8 ± 1.5	1.8 ± 0.6	2.2 ± 0.4
E C	1.4 ± 1.7	3.0 ± 1.8	2.5 ± 2.7	2.6 ± 1.4	2.7 ± 1.3
SD1 [ms]	105.8 ± 71.5	76.9 ± 36.6	114.8 ± 59.9	111.7 ± 64.1	74.5 ± 47.0
E C	116.6 ± 69.0	144.1 ± 147.0	139.0 ± 128.6	167.3 ± 246.0	224.8 ± 281.6

Relaxation 2. During Relaxation 1 the intensity of parasympathetic activity was similar to the Post-Intervention Baseline. The RMSSD under the control condition was higher compared to the experimental condition in each activity phase and according to standard deviation, was characterized by a high variability between individuals (again except during the Pre-Intervention Baseline phase).

The LF/HF ratio, representing the sympathovagal balance, reached the highest value during the Challenge in the experimental condition compared to the other sequences. In the control condition, the highest LF/HF score emerged during Relaxation 1 compared to the other phases.

Similar to RMSSD, the SD1 scores were overall higher in the control setting compared to the experimental setting.

As shown in Figure 5, the median cortisol concentration after a standardized EAT session (S4: 0.55 ng/ml) differed slightly from the sample prior to EAT (S1: 0.38 ng/ml) and at the beginning of an EAT session (S2: 0.33 ng/ml). During the EAT session, the median cortisol concentration reached a maximum value (S3: 1.21 ng/ml). The control condition revealed a lower median cortisol concentration for S1 (0.35 ng/ml), S2 (0.27 ng/ml) and S3 (0.31 ng/ml) but showed an increase post session in S4 (0.87 ng/ml), where the horses were already back in the stable.

Outcomes of EAT on the human participants have been reported elsewhere (Naber et al., 2019). Individual horses' HR, HRV (see Table A1) and cortisol responses (see Table A2) are provided in the Appendix.

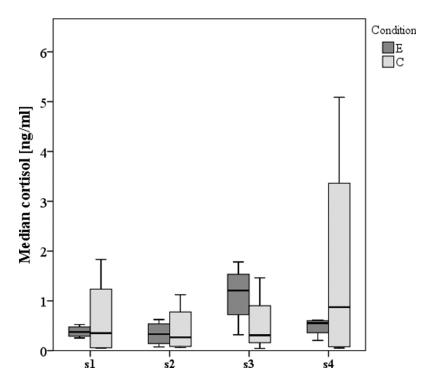


Figure 5. Boxplots of salivary cortisol concentrations of horses (N=4) prior to (S1), at the beginning (S2), during (S3) and shortly after (S4) the experimental condition (E) and the control condition (C).



Discussion

This study adds to the limited body of knowledge on equine cardiovascular and neuroendocrine responses to EAT delivered to young women with an intellectual disability. Therapy horses participated in standardized EAT sessions and a control condition without clients, in which they were solely guided by the therapist. The control condition was therefore similar to a regular training session, in which therapy horses commonly interact with therapists to maintain their skills and learn new tasks. A component of the training program is audible exhalation to provide horses with the opportunity to communicate their inner states during EAT (Naber et al., 2023). Clearly, this is a pilot study based on the responses of four horses using descriptive statistics and therefore, any changes that we have observed indicate fluctuations in physiological parameters rather than demonstrate differences that allow to draw further conclusions. The most important finding was that horses' autonomic and endocrine measurements during standardized EAT sessions revealed no signs of acute distress when experimental data were compared to control sessions. Higher levels of HR and cortisol during the Challenge in experimental sessions may suggest a greater physical demand caused by carrying the recipient on horseback. Data by Arrazola and Merkies (2020) point at an increase in equine heart rate and decrease of socio-positive behaviors over the course of repeated EAT sessions in a 10-week-program. In a study by Suthers McCabe and Albano (2004), p. 6 out of 33 horses had increased cortisol levels post EAT. The spectral HRV index LF/HF revealed a peak during the Challenge in the experimental condition. These results are in line with findings by Mendonça et al. (2019) who reported increased LF/HF values in the active working phase compared to preparation phase. Similarly, in comparison to an anticipatory phase prior to EAT or while clients sat on horseback, horses' cardiac and respiratory tone decreased during relaxing human-animal interactions on the ground (Ayala et al., 2021). In the absence of any activity-related changes in equine heart rate, behavior or the secretion of plasma adrenocorticotropin (ACTH, a pituitary gland hormone that is part of the HPA-axis pathway), serum and salivary cortisol were significantly lower after hippotherapy compared to after beginner class. Associations between HPA-reactivity and emotional arousal have been widely described (Fiksdal et al., 2019; Wirth et al., 2011). In horses, higher excitability has been previously related to increased cortisol responsiveness (Bohák et al., 2017).

Variations between heartbeats represent the interplay between the parasympathetic and the sympathetic nervous system, which is parallelled by HRV parameters. However, these physiological changes have implications also for mental and emotional well-being (Siepmann et al., 2008). Social interactions impact the cardiovascular system with conflicting encounters raising the risk for cardiovascular diseases. In contrast, positive social interactions that strengthen social support and coherence have been linked to a lower risk of coronary events (Cornelius et al., 2018). A sense of social coherence between individuals has been suggested to mutually unfold during nonverbal interactions with horses (Scopa et al., 2019; Zink & Deimel, 2019). The autonomic nervous system responds efficiently to physical, mental, and environmental stimuli in humans and horses as well (De Santis et al., 2017; Drinkhouse et al., 2012). As HRV fluctuates with psychological and environmental influences, it represents the organism's adaption and regulatory capacity (McCraty, 2017). As our measurements reflecting the overall variability (STDRR) as well as the intensity of parasympathetic activity were highest during the Challenge in EAT and the cortisol concentration quickly declined after EAT, the results do not suggest that the horses perceived the therapeutic service as substantially straining. Still, we observed higher RMSSD and SD1 scores, which both represent parasympathetic tone (Ciccone et al., 2017), for all phases in the control condition when the horses solely interacted with the therapist. These findings suggest a balanced psycho-physiological functioning which underlines the importance of including such regular therapist-horse interactions into the routine of therapy horses to further deepen the horse-handler familiarity and relationship. Similar results were reported by Scopa et al. (2020) who also proposed that high familiarity with the handler can shift horses' autonomic responses during social interactions.

Comparing the end of an EAT session to the beginning, the equine HR was slightly higher accompanied by a decrease in HRV measures. Accordingly, the autonomic activation caused by EAT might require additional time of rest until variables return to baseline. In the control condition, cortisol data revealed an increase post intervention, where the horses had already returned to the stable for 30 minutes and social encounters with conspecifics were possible to any extent. Also, the standard deviation suggests a considerable variability between individuals in the case of the control condition in post-session cortisol. Behavioral recordings to provide additional insights as to whether this increase was caused by interspecies social interactions would have been interesting to investigate the underlying causes. Another sequence of heart rate measurements at this time point could have yielded additional information on physiological arousal but was not scheduled in the protocol to protect both the horses and the heart rate monitor equipment once the horses returned to their herd. Similarly, control condition STDRR, RMSSD or SD1 (except for the pre-intervention baseline) was marked by high standard deviations, indicating higher individual variability that might even out if multiple control sessions per horse would have been considered. Accordingly, consecutive studies should explore also the phase of returning to the herd in more detail and include more than one control session per horse.

Although the EAT experience of the clients, therapist and horses, as well as the standardization of the subsequent phases (Pre- and Post-Intervention Baselines, Relaxation phases, Challenge) facilitated between-individual comparisons (Naber et al., 2019), a follow-up assessment with a bigger samples size would be needed for a more holistic interpretation of the findings. More specifically, it would be essential to also assess horse-directed actions and behavior from clients and therapists. In animal welfare assessment, behavioral measures such as locomotor activity but also stress-related gestures would be desirable to complement the physiological data (De Santis et al., 2017; Ferlazzo et al., 2023). Behavioral monitoring can be carried out during EAT but may also be interesting prior to and after sessions to gather data on the horses' anticipation and post-session activity. In the present study, lacking finances hindered a high-resolution camera set-up in the riding hall to monitor relevant behavioral changes in animals and humans. Accordingly, this study was planned as a pilot project to inform a larger scale follow-up study, in which a bigger sample and additional animal welfare indicators (i.e., respiration (Ayala et al., 2021), blood pressure (Ayala et al., 2021), behavior (Arrazola & Merkies, 2020; Ayala et al., 2021; Lansade et al., 2018; Mendonça et al., 2019; Merkies et al., 2018) and salivary oxytocin (Lansade et al., 2018; Malinowski et al., 2018)) shall be included. Similarly, it would be essential to apply the protocol used in this study to another cohort of clients that are commonly involved in EAT such as individuals diagnosed with PTSD (Malinowski et al., 2018; Merkies et al., 2018; Raman et al., 2023) or healthy controls (Fazio et al., 2013; Potier & Louzier, 2023) as the physiological response could be similar regardless of the rider not having a disability or having a different disability. Future studies should assess if the physiological responses are linked to EAT sessions or whether they parallel the activity that the horse is performing, regardless of the recipient. Apparently, the welfare of animals involved in psychotherapeutic protocols to improve human health remains an emerging area of research and ethical interest (Hediger et al., 2021). As suggested by Kelly et al. (2021), shifting the scientific perspective from the absence of negative affective states toward positive welfare indicators in equines during human-horse interaction is warranted.

Conclusions

The equine perspective of EAT is an emerging area of research. We conducted a pilot study using a standardized protocol to measure four horses' physiological welfare indicators during EAT sessions, which were planned to support a cohort of 10 young women with an intellectual disability. No acute signs of overload in the horses warranting reconsideration of the practice were detected when we compared our descriptive experimental data of HR, HRV, and/or salivary cortisol to values derived from control sessions in which the horses completed the same protocol but were solely



handled by the therapist. Accounting for the small sample size, more in-depth research is needed to draw any further conclusions.

Disclosure statement

No potential conflict of interest was reported by the authors.

Data availability statement

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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Table A1: Individual horses' HR, STDRR, RMSSD, LF/HF and SD1 during the experimental (E) and control (C) setting

:	:	-			RMSSD	!	:
Horse	Condition	Protocol phase	HR[bpm]	STDRR[ms]	[ms]	LF/HF	SD1[ms]
1	П	Pre-intervention baseline	31,027	102,803	123,804	1,866	87,835
-	U	Pre-intervention baseline	27,690	207,634	202,295	996′0	143,569
2	ш	Pre-intervention baseline	41,591	111,039	145,018	1,235	102,789
2	U	Pre-intervention baseline	45,353	40,383	39,970	3,996	28,326
٣	ш	Pre-intervention baseline	35,123	210,244	285,110	6,321	202,265
٣	U	Pre-intervention baseline	30,489	177,250	269,516	0,093	191,207
4	ш	Pre-intervention baseline	33,828	40,702	42,765	3,048	30,330
4	U	Pre-intervention baseline	33,988	185,050	145,453	0,721	103,158
-	ш	Relaxation 1	38,209	114,868	162,315	1,414	115,058
-	U	Relaxation 1	31,355	353,019	260,241	1,374	184,613
2	ш	Relaxation 1	54,097	106,698	125,426	1,480	88,850
2	U	Relaxation 1	45,746	42,482	38,441	5,481	27,242
3	ш	Relaxation 1	35,314	115,205	106,849	1,466	75,764
3	U	Relaxation 1	29,984	308,145	471,987	2,513	334,871
4	ш	Relaxation 1	34,284	34,522	39,235	2,367	27,825
4	U	Relaxation 1	30,318	32,682	41,691	2,431	29,580
1	ш	Challenge	46,840	184,835	205,980	4,029	145,961
1	U	Challenge	44,062	413,690	464,171	0,549	328,980
2	ш	Challenge	64,535	200,365	235,402	2,099	166,701
2	U	Challenge	68,448	202,171	146,456	2,094	103,731
3	ш	Challenge	43,910	143,835	163,872	2,787	116,134
3	U	Challenge	38,264	82,670	71,352	6,353	50,588
4	ш	Challenge	47,374	48,496	43,052	3,451	30,508
4	U	Challenge	38,778	100,729	102,296	0,973	72,524
1	ш	Relaxation 2	40,036	136,395	181,438	1,433	128,611
1	U	Relaxation 2	27,853	57,831	83,381	1,958	59,182
2	ш	Relaxation 2	52,969	104,464	142,479	1,208	100,927
2	U	Relaxation 2	44,326	39,325	39,592	4,457	28,059
3	ш	Relaxation 2	36,054	191,800	262,060	2,121	185,766
3	U	Relaxation 2	59,969	478,763	755,190	1,275	535,801
4	ш	Relaxation 2	33,763	36,567	44,530	2,363	31,583
							:

SD1[ms] 127,497 200,699 99,861 29,272 41,518 630,471 29,097 38,888 46,037 LF/HF 2,845 1,520 0,943 2,504 4,144 2,348 2,402 2,308 179,850 282,816 140,960 41,304 58,539 888,430 RMSSD [ms] 64,889 41,021 54,809 STDRR[ms] 54,910 134,388 237,690 113,308 40,404 50,679 594,019 33,488 HR[bpm] 30,149 37,209 28,152 51,228 45,065 33,985 29,054 30,016 32,375 Post-intervention baseline Protocol phase Relaxation 2 Condition Horse

Table A1: (Continued).

Table A2: Individual horses' cortisol responses during the experimental (E) and control (C) setting

Horse	Condition	Sample Number	Cortisol [ng/ml]
1	E	S1	0,428
1	C	S1	0,638
2	E	S1	0,323
2	C	S1	0,051
3	E	S1	0,255
3	C	S1	1,832
4	E	S1	0,525
4	C	S1	0,064
1	E	S2	0,624
1	С	S2	0,062
2	Е	S2	0,212
2	С	S2	1,123
3	Е	S2	0,075
3	C	S2	0,108
4	Е	S2	0,451
4	C	S2	0,429
1	Е	S3	1,127
1	C	S3	0,044
2	Е	S3	1,782
2	C	S3	0,276
3	E	S3	0,320
3	C	S3	0,343
4	Е	S3	1,287
4	C	S3	1,462
1	Е	S4	0,205
1	C	S4	0,052
2	E	S4	0,589
2	C	S4	0,107
3	E	S4	0,616
3	С	S4	1,638
4	E	S4	0,517
4	С	S4	5,088