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Heart rate and salivary cortisol as indicators of arousal and synchrony in clients, therapy horses and therapist in equine-assisted therapy

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ABSTRACT

Background: This exploratory study aimed to analyse physiological interaction processes in equine-assisted-therapy (EAT) between client, therapy horse and therapist.
Methods: We measured heart rate (HR), heart rate variability (HRV) and cortisol levels before, during and after a standardized therapy session and a control condition in one therapist, four therapy horses and ten female clients in emerging adulthood (Mn = 21.8 years, SD = 3.39). The clients were diagnosed with mild (N = 5) to moderate (N = 5) intellectual disability (ID).
Results: There was no significant change in the client's HR, HRV and cortisol levels during an EAT session. No difference was observed between therapy sessions with or without a therapy horse, except during the challenge phase of the EAT protocol, where clients had a significantly lower HR when interacting with the therapy horse. HR between therapist and client correlated significantly, as well as between therapist and horse. This effect was greater when therapists interacted with a familiar horse. Clients' and horses' HRs also correlated, but only when the horse was the clients' familiar and preferred horse.
Conclusions: These results indicate that relationship intensity is an important factor for the synchronization process. Moreover, the inclusion of horses in a therapeutic setting can lead to a decreased HR in young adults with intellectual disability while mastering a challenge. Future research should investigate this potential benefit of EAT, considering the reciprocal influences and the relationship between client, therapist and horse.

1. Introduction

1.1. Stress reduction in equine-assisted therapy (EAT)

An emerging body of research has investigated effects of the human-horse interaction suggesting that interaction with animals can improve human wellbeing and health [1–10]. Equine-assisted therapy (EAT) is considered a promising practice as a complementary treatment for individuals with intellectual disabilities [11,12]. Persons with intellectual disabilities have an impaired intellectual and adaptive functioning [13], impacting various skills. Emerging adulthood, a time of major changes, challenges, uncertainties, and exploration, represents a period of

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"feeling in between" – individuals at this age have left the dependency of childhood and adolescence behind but have not yet fully entered the world of adulthood with its ongoing obligations [14]. Due to a greater dependence on their families and fewer career opportunities [15], this vulnerable phase may be especially stressful for youth with intellectual disabilities [16]. Stress regulation plays a key-role in maintaining mental and physiological health [17]. Strengthening individual resilience through interventions that are cognitively and emotionally stimulating and support stress reduction is particularly needed during this transition into adulthood [15,16,18]. While EAT is often advocated for this population, its efficacy in reducing stress remains unclear [19].

While studies provide evidence for increased motor control due to EAT [20–22], the psychological benefits and underlying mechanisms of EAT are not yet fully understood [12]. The fact that horses exhibit sophisticated non-verbal communication when interacting not only with conspecifics but also with humans [23] is essential for people with intellectual disabilities, as they often struggle to use verbal language appropriately and therefore frequently feel misunderstood [24]. Interaction and the ability to influence the environment are key factors not only in communication but also in connection with self-efficacy, which is associated with higher well-being [12]. Self-efficacy is a basic human need as well as a form of self-expression [25]. Being able to control a situation is one of the most effective stress-reducing factors [26].

Social interactions impact the cardiovascular system. Conflicting social interactions are risk factors for cardiovascular diseases, while positive social interactions, such as social support and belonging, seem to predict a lower risk of coronary events [27]. The autonomic nervous system (ANS) responds to physical, mental and environmental stressors in both humans and animals [28,29]. It consists of the sympathetic and parasympathetic branches [30], which regulates variations in cardiovascular response [31]. For example, there can be an increase in heart rate (HR) during pleasant social interactions in humans with high emotional reactivity [27]. General stress indicators related to nervous system arousal are associated with increasing concentrations of the hormones cortisol and adrenaline, as well as higher HR [17] and decreased heart rate variability (HRV) [32]. HRV shows the variation between heartbeats and represents the interplay between the parasympathetic and sympathetic nervous system [28,33]. Due to its fluctuations with psychological and environmental influences, HRV is a measurement of the organism's adaption and regulatory capacity [34]. HR and HRV are therefore useful indicators to investigate a potential stress-buffering effect of EAT for individuals with intellectual disabilities.

The first aim of the study was to investigate these potential stress-buffering effects of EAT. The initial investigation examined how a standardized EAT session influences HR, HRV, and salivary cortisol in young adults with intellectual disabilities.

1.2. Synchronization in EAT

During social interactions, the coordination of nonverbal behaviors such as facial expression, body posture, or vocalization can result in interspecific synchronization including emotional contagion, joint attention, or synchronous behavior in many mammalian species [35–39]. Synchrony enhances cooperation, social connection, affectivity, and partner perceptions in humans [40]. Among living beings, (behavioral) synchronization is widespread and even occurs across species [36,41,42]. The experience of synchrony originates in the mother-child relationship, and high levels of synchrony have been associated with efficient bonding and stress reduction [43,44]. Interactions with a high level of synchronization are more efficient, even across species [41,45]. Synchronization is crucial to our well-being and positively impacts human-horse interventions [46].

The effectiveness of EAT may be explained by the ability of horses to interact not only physically but also emotionally with humans. Emotional transmission and connection, along with the health-supporting effects of touch and physical proximity may lead to interactions within a synchronized systemic hetero-species relationship [47]. Emotional transmission and synchronization are essential for all social interactions, as all creatures strive for a sense of coherence [48]. The mechanism of coherence involves rhythmic synchronous patterns of cardiac activity that lead to the subjective activation of distinct emotional states and improved cognition [34,49]. The harmonization of heart rate further connects with breathing and brain waves, mediated by the interplay of efferent and afferent fibers of the sympathetic and parasympathetic branches of the ANS [34]. A sense of coherence denotes an individual's ability to perceive a coherent connection with themselves and their social environment. Such a sense of coherence resulting from synchrony between individuals has been suggested to unfold in humans during nonverbal interactions with horses [46,47]. Therefore, horses could be helpful partners in improving synchronization, the feeling of coherence and connection, and consequently, enhancing the well-being of individuals with intellectual disabilities.

To fully understand the processes of synchrony within the client-therapist-horse triad in EAT, it is crucial to investigate all involved participants. However, previous research has typically measured markers of arousal only in the clients [50–53], only in the horses [54–61], or in both the horses and clients [29,62–66] as well as horses and riders [67], horses and humans [68] with positive or negative attitude toward companion animals [69] and horses and humans with different stress burdens [70]. To date, there is one pilot study assessing also the EAT professional besides the clients and the horses [71], but it could not investigate the therapist-client-horse triad team because some recordings from the client and the horse were not readable. Addressing all three actors within the client-therapist-horse triad is important in the context of the One Health framework [72], which emphasizes the inextricable link and reciprocal processes between humans, animals and their environment.

Therefore, the second aim of this study was to investigate all three participants of the triad in EAT and to analyse synchronization of HR, HRV and salivary cortisol between clients, horses, and therapist during a standardized EAT session.

2. Materials and methods

2.1. Participants

2.1.1. Clients

Inclusion criteria included experience in EAT, female gender, a diagnosed intellectual disability, interest in the human-horse-interaction, and an age range covering emerging adulthood. Exclusion criteria were acute psychosis, intake of medications that affect neuroendocrine cascades and autonomic rhythmicity, and age below 16 years or above 30 years.

All therapists from the EAT center were informed about the research project and inclusion criteria. They provided contact details of potential clients or their caregivers to the research team. Potential participants were contacted by telephone and informed about the planned study, including the possibilities and requirements for participation. After a week of reflection, they were contacted again to discuss and agree upon possible times for the survey. Fifteen potential participants who met the criteria were contacted. Due to the requirement to appear four times at the same time of day within two weeks, five potential participants were unable to take part. For two participants, working hours conflicted with the research timetable, one was on vacation for part of the time, and for two, it was impossible to attend, and there was no option for a delivery service. This led to a final sample of ten participants.

Ten young women, aged between 16 and 27 years (Mn = 21.8, SD = 3.39), participated in the study. They had between five and 19 years of previous experience in EAT (Mn = 12.8, SD = 4.71) and were diagnosed with mild (N = 5) to moderate (N = 5) intellectual disability according to the Glasgow Level of Ability and Development Scale (73) . In the demographic questionnaire, clients could indicate their favorite horse, which they had known for years and with which they had regular ongoing therapy sessions. If the client's favorite horse was one of the four chosen horses for the study, the client was assigned to her favorite horse (N = 5). If the favorite horse was not available, another horse was selected at random (N = 5). This schedule was arranged in line with the resources and requirements of the EAT facility and to select two group of clients with two different levels of relationship intensity with the horse.

2.1.2. Horses

Two mares and two geldings (N = 4) participated in the study. All four horses were of the Criollo breed, with an average age of 14.8 years (SD = 5.6) and an average of 9.4 years (SD = 6.6) of working experience in the therapy setting. All horses underwent regular veterinary checks (more than twice a year), and there were no known diseases, particularly those affecting cortisol release and heart activity, which were controlled for prior to enrollment in the study. None of the horses were on any medications or exhibited any abnormal behavior. The horses lived in a herd of twenty therapy horses in a spacious open stable. The area included four indoor sleeping areas, a large sandy area, trees and fences for places to retreat and separated areas. Several feeding areas for hay, water, and minerals were evenly distributed. Their everyday life consisted of a balanced mix of EAT sessions, therapy horse training in the form of muscle training, gymnastic exercise, and supervision, as well as break times and leisure activities to restore their inner balance.

2.1.3. Therapist

The female psychotherapist, aged 37, had 21 years of experience in EAT. She was in good clinical health, free of cardiovascular diseases, and not taking any medication. The therapist knew the clients by sight but had no previous interaction or relationship with them. The horses in the study were either very familiar (N = 2) or less familiar (N = 2) to the therapist. The familiar horses were those with which the therapist worked several times a week in therapy sessions. She also took care of balancing work, joint leisure activities, and training the horses for therapy purposes. The therapist knew the other horses from individual therapy sessions but did not conduct any activities with them apart from therapeutic work. The rationale for including only one therapist in this study was to minimize any confounding effects related to the personality, horse handling style, or therapeutic guidance. Moreover, similar to the selection of client-horse dyads, we sought to include two levels of relationship intensity (high and low familiarity) between therapist and horses, which could be fulfilled with the present protocol. An overview on the measurements and the number of EAT and control sessions that clients, horses and the therapist participated in is provided in Fig. 1.

2.2. Study design and procedure

Data collection took place from February 12th to 25th 2018, at an EAT center in Vienna, Austria. Clients received *Equotherapy*, a specialized form of EAT that focuses on nonverbal communication and utilizes particularly well-trained therapy horses to respond to the nonverbal impulses from humans. By integrating these cues, therapists can draw conclusions about the psychological and emotional state of a client and expand therapeutic strategies [46,78]. The EAT center offers therapeutic support for individuals dealing with grief, trauma and palliative care [79].

2.2.1. Measurements

An overview on the sampling schedule and the number of EAT and control sessions that clients, horses, and therapist participated is shown in Fig. 1. This study was designed as a controlled, randomized trial with repeated measurements. Each of the ten clients underwent four standardized therapy sessions: two sessions with the therapy horse and two control sessions. Both conditions consisted of the same exercises guided by the therapist and conducted either with a horse or a barrel horse. Data collection took two weeks, with clients participating in two sessions per week. Allocation to experimental or control condition was determined randomly in advance by tossing a coin. However, to maintain motivation, the first session for each participant took place with the horse, while the subsequent three sessions were randomly assigned until each client experienced each condition twice. Each of the therapy horses participated in 2x10 EAT sessions under the conditions of familiar or unfamiliar client and familiar or less familiar therapist. The therapist guided 2x10 EAT sessions with two familiar and two less familiar horses in the experimental condition. In the control condition, the therapist conducted 2x10 sessions.

2.2.2. Infrastructure and sampling schedule

All EAT sessions took place in a closed riding arena between 1:30 to

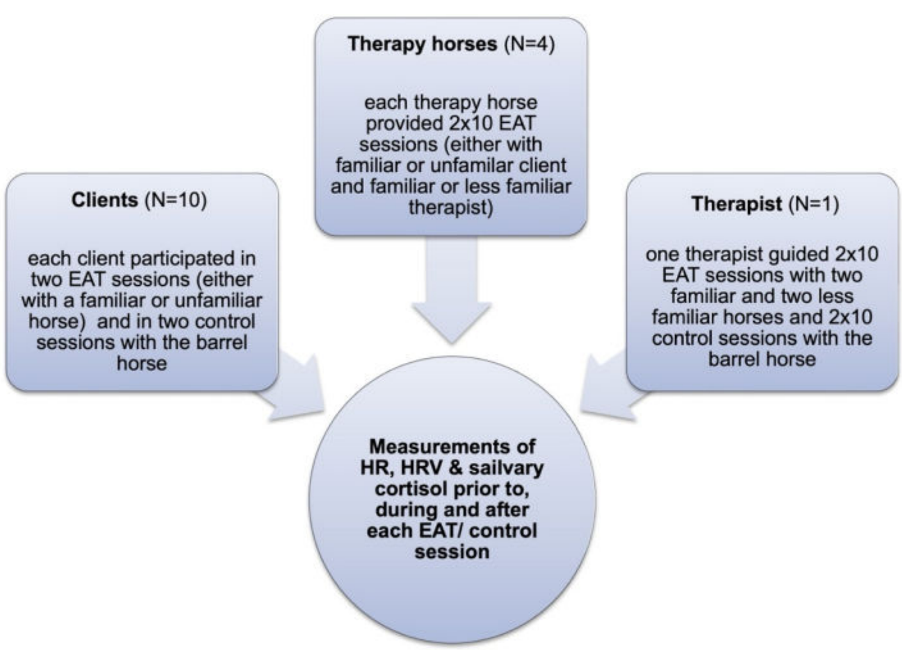

Fig. 1. Number of sessions and overview on the measurements.

5:30 p.m., with an average ambient temperature of 2.5 °C (±1.8). The infrastructure in the riding arena remained unchanged throughout the entire duration of the study to ensure that the measurements were carried out as standardized as possible. In the riding arena (20 × 40 m), there was a ramp for mounting and dismounting the horse, two chairs, the barrel horse, the obstacle course, and a table on which the technology was set up (see Fig. 2). 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. The responsible person at the experimenter table monitored the time intervals according to the study sequences (i.e. starting the HR measurements simultaneously to ensure that the required 5 min of each phase are adhered to and collecting all saliva samples; see Fig. 3).

Every session consisted of five phases: *Pre-Intervention Baseline*, *Relaxation 1*, *Challenge*, *Relaxation 2* and *Post-Intervention Baseline* (see Fig. 3).

The time schedule of the study protocol is depicted in Fig. 3. Upon arrival, clients entered the therapy center and waited for at least 15 min in the waiting area. To account for the endogenous circadian rhythm, each study participant was scheduled to visit at the same time of the day to ensure the comparability of the four measurement time points [32, 80]. The participants were required to fast for 90 min prior to data collection for both the cortisol sampling and the HRV measurement. Since the activity prior to an HRV assessment is critical to the results, a short break or quiet rest before the measurement is desirable [80]. This was ensured by waiting at least 15 min after arriving at the therapy center before the measurements started in the riding arena. Then, the belt for the heart measurements was adjusted according to the manufacturer protocol. The clients were then introduced to the therapist and

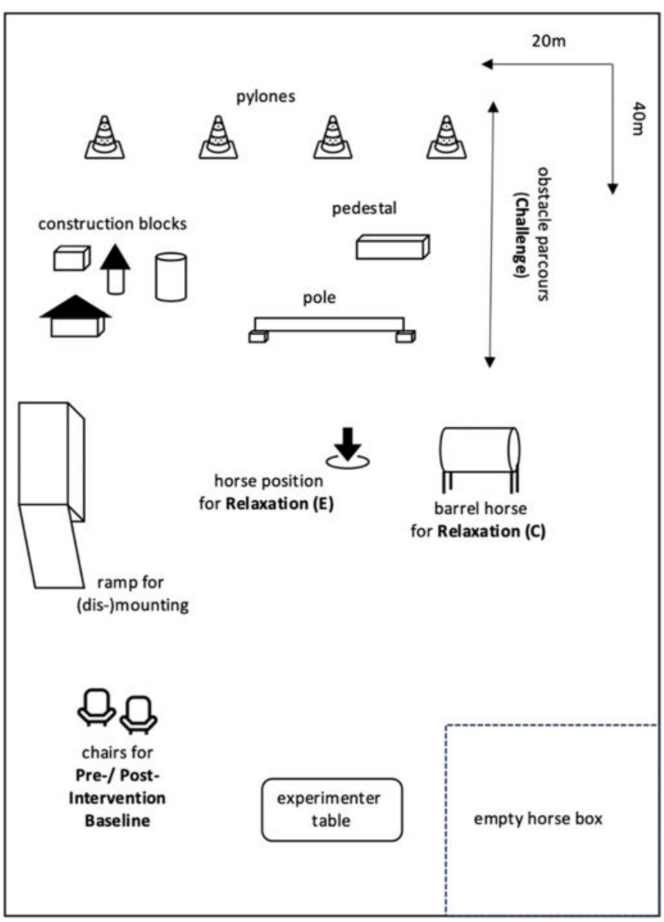

Fig. 2. Structure of the riding arena and organization chart for the study protocol.

brought to the riding arena, where the process was explained according to the study protocol.

2.2.2.1. Pre-intervention baseline. In the experimental condition, the horse was greeted and then the first saliva sample was collected from therapist, client and horse. Next, the therapist and client rested on chairs (see Fig. 2), and HR assessment was initiated simultaneously for the horse, therapist and client to start the pre-intervention baseline measurements.

During the trials with the horse, the pre-intervention baseline measurement consisted of 5 min, during which the client and the therapist sat on chairs, and the horse stood next to them. In the control condition, the client and therapist sat on the chairs without a horse next to them. Clients were instructed to sit still and avoid moving while adjusting to the environment. Conversations with the therapist were possible at all times and were initiated by most of the clients.

2.2.2.2. Mounting/walking. Between the pre-intervention baseline and the relaxation phase of the experimental condition, the client mounted the horse and sat on horseback while the therapist walked with the horse two rounds in the riding arena. In the control condition, the therapist and client walked two rounds on feet. Accounting for individual gait speed and preferences of horses and clients, this phase was not time-restricted. However, it served as a condition to control for effects of movement (riding versus walking) on HR reactivity for the following HR recording interval, which was initiated directly after the completion of the two rounds.

2.2.2.3. Relaxation 1. In the experimental condition, the horse remained standing calm in the same place, and the therapist instructed a relaxation exercise for 5 min while the client remained sitting on horseback. The clients could choose whether they preferred a breathing exercise, a visualization exercise, a relaxation exercise for individual body parts, or a quiet time on the horse without any instructions. In the control condition, relaxation exercises took place with the clients sitting on a barrel horse, a wooden horse used for vaulting. The positions of the horse or barrel horse are depicted in Fig. 2.

2.2.2.4. Challenge. After the pre-intervention baseline, the challenge phase began, during which the client was asked to master a challenge. On horseback, the client independently navigated an obstacle course for 5 min, receiving support from the therapist according to their individual riding skills. The obstacle course (see Fig. 2) consisted of four pylons, large, colorful construction blocks, a pedestal, and a pole. In the experimental condition, clients were instructed to ride a slalom on the horse between the pylons, find a path between the blocks or ride a circle around each, climb onto the pedestal, and step over the pole. In the control condition, clients were instructed to perform various exercises on foot, such as walking the slalom backwards, balancing over the pole, standing on the pedestal, and walking a specific path between the blocks.

After the challenge phase, a second saliva sample was collected from the horse, client and therapist while the client sat on horseback. In the control condition, a second saliva sample was collected from the client and therapist standing next to the obstacle course.

2.2.2.5. Relaxation 2. Then the second relaxation phase started. As in the previous relaxation phase, the horse stood calmly, and clients were guided by the therapist. In the control condition, clients sat on the barrel horse again.

2.2.2.6. Post-intervention baseline. The protocol ended in the experimental condition when the horse was dismounted. In both conditions, the client and therapist sat down again in the chairs for the post-intervention baseline measurements. They sat there for 5 min and

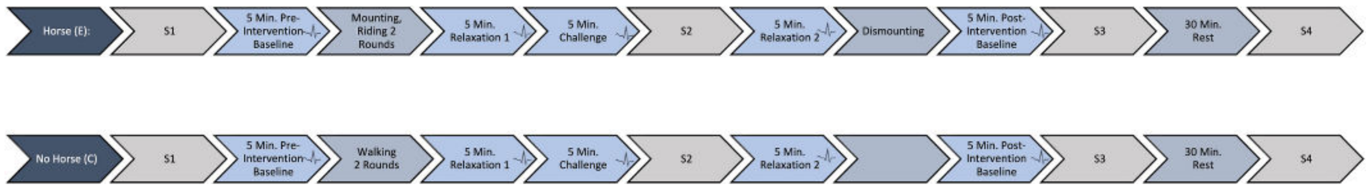

Fig. 3. Study *procedure of the standardized therapy session*
E = Experimental condition, C=Control condition; S1 – S4 = saliva samples; indicates 5 min intervals of HR recording.

were then briefed with the same instructions as in the pre-intervention baseline phase.

After that, the cardiac activity measurements were stopped simultaneously for the horse, the therapist, and the client, and the third saliva sample was collected while the therapist and client were still sitting in the chairs. Afterwards, clients were bid farewell and taken to a quiet room, where they could choose to draw or just sit quietly for another 30 min. Then, the final saliva sample was collected.

2.3. Research instruments

2.3.1. Questionnaire

Participants were asked to fill out a questionnaire that collected sociodemographic data such as age, gender, favorite horse, and experience in EAT. The Glasgow Level of Ability and Development Scale [73] was used to assess the individual requirements for support and the severity of the intellectual disability. This brief screening consists of five questions and evaluates a person's ability and development level through an external assessment. In this study, the screenings were completed by caregivers. The scale can be used to quickly assess a person's independence and need for support, thereby referencing the severity of intellectual disability (none, mild, moderate or severe). The questions address the need for support in areas such as eating and drinking, intimate hygiene, personal safety, communication, and decision-making [73].

2.3.2. Cardiovascular activity

Cardiovascular activity was recorded simultaneously in participating humans (clients and the therapist) and the therapy horse throughout the entire session with the Polar ® V800 telemetric system (Kempele, Finland). Humans wore a chest strap with the Bluetooth sensor, while therapy horses were equipped with an equine H7 HR sensor electrode base set. According, to the manufacturer's protocol, the negative electrode pad was placed on the left under the strap, and the positive one beneath the withers under the pad. The equine H7 belt can be easily adjusted to fit the horse's size. To ensure adequate data transmission, the horse's coat was wetted, and electrode gel was applied. Objective measures of autonomic activation included HR and HRV, specifically overall variability (SDNN) and parasympathetic tone (RMSSD, SD1).

For the analysis of cardiovascular activity data, the sequences of the therapy session from the therapist, the client, and the horse were divided into 5-min sections and analyzed using the *Kubios HRV* program (Kuopio, Finlan) [74]. HRV was manually corrected for artifacts via Kubios, which is considered the most valid method for controlling artifacts [75].

2.3.3. Salivary cortisol

Adrenocortical reactivity was assessed via salivary cortisol sampling. As demonstrated in Fig. 3, four saliva samples (pre- and post-exposure) were taken under both conditions (horse and barrel horse). The first measurement was taken at least 15 min after the participant's arrival (S1), the second after the challenge phase (S2), the third after the end of the standardized therapy session (S3), and the fourth after a 30-min rest phase (S4), during which clients spent time sitting quietly and drawing in the therapy room. Commercial cotton swabs (Salivette®, Sarstedt, Wiener Neudorf, Austria) without any saliva-stimulating additives were used to determine salivary cortisol concentrations. Participants were thoroughly instructed on how to collect their own saliva sample. They placed the swab into their cheek pouch and allowed it to saturate with saliva for approximately 60–80 s. Afterwards, the swabs were placed back into the device container and immediately frozen at −20 °C.

Additionally, control measurements of cortisol concentrations at home were scheduled at three different time points (7:00–9:00, 12:00–14:00, 18:00–20:00) on a day without any therapy (EAT or other therapy) or leisure time activity for all clients. The study provider ensured that all saliva samples were stored and cooled at −20 °C immediately after sampling at the therapy center and then transferred to the laboratory. Prior to analysis, samples were carefully defrosted on ice and centrifuged at room temperature for 15 min at 3000×*g*. Cortisol in saliva was determined using a highly sensitive enzyme immunoassay [76,77].

In the laboratory, a total of 10 μl of a clear saliva dilution (1 + 9 with assay buffer) was used and all samples were assayed in duplicates. Average intra-assay and inter-assay coefficients of variance were less than 10 % and 15 %, respectively. Due to low saliva sample volumes, the samples collected at 12:00–14:00 and 18:00–20:00 were pooled, and the respective values were averaged.

2.4. Statistics

All data were analyzed using *IBM SPSS Statistics 24* [81]. Due to the probability of error, the significance level was set at $\alpha = 5$ % and results with $p < 0.050$ were therefore assessed as significant.

2.4.1. Physiological parameters over the course of EAT

To examine changes in the physiological parameters HR and HRV over the course of an EAT, the mean HR, SD1, SDNN and RMSSD of the first measurement *Pre-Intervention Baseline* of clients were compared to the *Post-Intervention Baseline* in the experimental condition with the horse. To analyse changes in the cortisol levels over the course of an EAT, the first saliva sample *S1* before EAT was compared to the saliva sample *S4* after EAT.

Data were inspected for normal distribution with the Kolmogorov-Smirnov-Test. HR and HRV (SD1, SDNN, RMSSD) were normally distributed. Thus, a dependent *t*-test was carried out. Cortisol concentrations were not normally distributed, and a Wilcoxon signed-rank-test as conducted to compare the first (S1) and last (S4) salivary samples. Mean and SD were used. The significance was one-tailed, because we predicted Mean HR and cortisol to be lower at *Post-Intervention Baseline* and Mean HRV (SD1, SDNN, RMSSD) to be higher. Pearson's correlation coefficient *r* was used as the effect size, calculated with $r=\sqrt{(t^2/(t^2+df))}$ for HR, SD1, SDNN, and RMSSD and $r=z/\sqrt{N}$ for cortisol.

2.4.2. Condition effect on physiological parameters during the five intervention phases

To analyse the patterns of HR, HRV, and cortisol level during EAT, the measurements of these parameters during the experimental condition with the horse were compared to the control condition without the horse. The effect of the condition on HR, SD1, SDNN and RMSSD was analyzed by comparing the horse condition with the control condition for all five periods *Pre-Intervention Baseline*, *Relaxation 1*, *Challenge*, *Relaxation 2,* and *Post-Intervention Baseline*. The effect of the condition on salivary cortisol concentrations was analyzed by comparing the measures after the experimental condition (E-S4), after the control condition (C-S4), and at home (H).

Data were inspected for normal distribution with the Kolmogorov-Smirnov-Test. HR was normally distributed during all five periods of measurement in both conditions. Thus, a dependent *t*-test was carried out with the factor experimental versus control to assess the effect of the horse on HR for each of the five phases. The HRV parameters SD1, SDNN, and RMSSD were not normally distributed. Therefore, Wilcoxon-Signed-Rank tests were used to compare the experimental with the control condition for all five phases. Cortisol concentrations were also not normally distributed and a Friedman test was used to compare the experimental and control conditions. Mean and SD were used. The significance for HR, SD1, SDNN, and RMSSD was one-tailed, because we predicted Mean HR to be lower in the experimental condition and Mean HRV (SD1, SDNN, RMSSD) to be higher. To avoid an alpha-error accumulation in the multiple comparisons for the analysis of HR, SD1, SDNN, and RMSSD during all five phases in both conditions, a Bonferroni correction was applied, and results with $p < 0.010$ were defined as significant. For cortisol, there was no prior assumption about the direction of the effect, so the significance was two-tailed. Pearson's correlation coefficient r was used as the effect size, calculated with $r=\sqrt{(t^2/(t^2+df))}$ for HR and $r=z/\sqrt{N}$ for SD1, SDNN, and RMSSD.

2.4.3. Synchronization between horse, client and therapist

To analyse the synchronization, HR, SD1, SDNN, RMSSD and salivary cortisol of the experimental condition were examined for correlations during each of the five periods *Pre-Intervention Baseline*, *Relaxation 1*, *Challenge*, *Relaxation 2,* and *Post-Intervention Baseline,* between horse and client, therapist and client, and therapist and horse.

To analyse the amount of synchronization between horse, client, and therapist, in HR and HRV parameters as well as cortisol, non-parametric Spearman correlations were used because the data were not normally distributed and the scatterplot showed no linear relation. The correlation coefficient *r* was used for the calculations of correlations as well as for the effect sizes, and the significance level was two-tailed.

For explorative investigation, the effect of familiarity and the relationship between humans and horses was examined. The data set was divided into unfamiliar and familiar horses for the therapist, as well as favorite and unfamiliar horses for clients. Correlations were calculated to investigate the influence of relationship on the synchronization.

3. Results

3.1. Physiological parameters over the course of EAT

The results showed no significant change in HR, HRV, and cortisol level from the first to the last measurement of an EAT session (experimental condition with the horse), (Table 1). The descriptive data of HR and salivary cortisol indicate higher values at *Pre-Intervention Baseline* and *S1* compared to *Post-Intervention Baseline* and *S4*, suggesting a nonsignificant decrease in HR and cortisol with a medium effect. Descriptive interpretation of HRV (SD1, SDNN, RMSSD) indicates higher values at *Post-Intervention Baseline* than at *Pre-Intervention Baseline.*

3.2. Condition effect on physiological parameters during the five intervention phases

3.2.1. HR

There was no statistical difference for HR in each of the *Pre-Intervention Baseline* ($t(19) = 0.60$, $p = 0.277$, $r = 0.14$), *Relaxation 1* ($t(19) = -0.36$, $p = 0.361$, $r = 0.08$), *Relaxation 2* ($t(19) = -1.47$, $p = 0.079$, $r = 0.32$), or *Post-Intervention Baseline* ($t(19) = -0.75$, $p = 0.0232$, $r = 0.17$) between the experimental and control conditions (see Fig. 4). During *Challenge*, a significant difference with a large effect was found between the HR in the experimental condition with a horse ($M = 88.10$, $SD = 14.67$) and the HR in the control condition ($M = 103.52$, $SD = 19.05$) ($t(19) = -5.569$, $p < 0.001$, $r = 0.78$; see Fig. 4).

Table 1
Results of the physiological parameters at start and at end of EAT.

	Pre- Intervention Baseline	Post-Intervention Baseline	t (19)	*p*	N	*r*
	Mean physiological parameter (SD)	Mean physiological parameter (SD)				
HR	76.7 (14.2)	75.1 (14.4)	1.16	0.131	20	0.26
SD1	44.1 (23.0)	54.1 (40.0)	−1.29	0.107	20	0.28
SDNN	63.9 (36.2)	73.4 (44.5)	−1.15	0.133	20	0.26
RMSSD	62.3 (42.3)	76.4 (56.5)	−1.29	0.107	20	0.28
	S1	S4	z	*p*	N	*r*
	Mean physiological parameter (SD)	Mean physiological parameter (SD)				
Coritsol	16.6 (23.4)	10.0 (16.4)	−1.37	0.091	18	−0.32

HR = heart rate, SD1 = standard deviation of the short-term N-N interval variability (parasympathetic tone), SDNN = standard deviation of the N-N intervals (overall variability), RMSSD = root mean square of successive differences between normal heart beats (parasympathetic tone).
SD = standard deviation, t = t-value (dependent T-test), *p* = probability value for significance, N = sample size, *r* = Pearsons' correlation coefficient for effect size, z = z-value (Wilcoxon signed-rank-test).

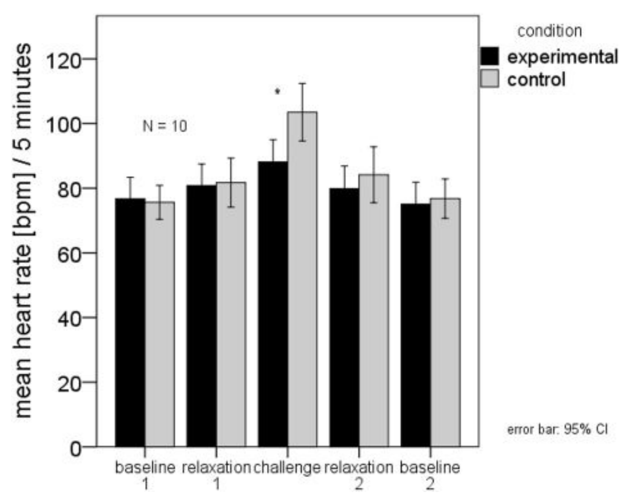

Fig. 4. Mean heart rates of clients during experimental (with horse) and control (without horse) sessions.

3.2.2. HRV

There was no significant difference in SD1, SDNN, and RMSSD between the two conditions across all five phases (see Table 2).

3.2.3. Cortisol

Results showed no significant differences in cortisol concentrations between the experimental condition, the control condition, or at home (X^2 (15) = 2.13, $p = 0.344$). Descriptive data indicates that the lowest cortisol concentrations were at home ($M = 3.0$, $SD = 4.0$) and that concentrations in the control condition ($M = 22.0$, $SD = 36.5$) were not significantly higher than in the experimental condition ($M = 9.4$, $SD = 17.7$).

Table 2
Comparison of SD1, SDNN and RMSSD for all phases between the experimental and control condition.

	Mean SD1 Experimental (SD)	Mean SD1 Control (SD)	*z*	*p*	N	*r*
Pre-Intervention Baseline	42.1 (31.7)	44.1 (23.0)	−0.11	0.456	20	−0.02
Relaxation 1	39.1 (33.1)	34.8 (21.6)	−0.93	0.176	20	−0.21
Challenge	32.5 (34.0)	23.9 (20.0)	−0.86	0.196	20	−0.19
Relaxation 2	41.4 (38.6)	57.4 (114.4)	−1.27	0.102	20	−0.28
Post-Intervention Baseline	41.4 (38.6)	57.4 (114.4)	−1.27	0.102	20	−0.28
	Mean SDNN Experimental (SD)	**Mean SDNN Control (SD)**	***z***	***p***	**N**	***r***
Pre-Intervention Baseline	63.9 (36.1)	63.6 (37.6)	−0.34	0.369	20	−0.08
Relaxation 1	51.5 (25.1)	58.5 (42.2)	−1.01	0.157	20	−0.23
Challenge	38.7 (23.7)	41.4 (33.8)	−0.22	0.412	20	−0.05
Relaxation 2	95.0 (197.1)	61.0 (45.7)	−0.56	0.288	20	−0.13
Post-Intervention Baseline	73.4 (44.5)	71.2 (62.8)	−0.37	0.355	20	−0.08
	Mean RMSSD Experimental (SD)	**Mean RMSSD Control (SD)**	***z***	***p***	**N**	***r***
Pre-Intervention Baseline	62.3 (42.3)	59.5 (44.7)	−0.11	0.456	20	−0.02
Relaxation 1	49.2 (30.5)	55.2 (46.8)	−0.93	0.176	20	0.21
Challenge	33.7 (28.3)	46.0 (48.0)	−0.86	0.196	20	−0.19
Relaxation 2	81.1 (161.5)	58.5 (54.5)	−1.31	0.096	20	−0.29
Post-Intervention Baseline	76.4 (56.5)	70.1 (74.7)	−0.64	0.263	20	−0.14

HR = heart rate, SD1 = standard deviation of the short-term N-N interval variability (parasympathetic tone), SDNN = standard deviation of the N-N intervals (overall variability), RMSSD = root mean square of successive differences between normal heart beats (parasympathetic tone).
SD = standard deviation, z = z-value (Wilcoxon signed-rank-test), p = probability value for significance, N = sample size, r = Pearson's correlation coefficient for effect size.

3.3. *Synchronization between horse, client and therapist*

3.3.1. *Synchronization between horse and client*

The results on synchronization showed no significant relation between HRV, cortisol concentration, and HR of the horse and client during the entire standardized EAT session. However, as seen in Fig. 5, the analysis revealed a significant correlation between the HR of clients and horses when the interaction took place with a familiar horse ($r = 0.38$, $p = 0.007$) compared to no significant correlation when clients were paired with an unfamiliar horse ($r = -0.06$, $p = 659$).

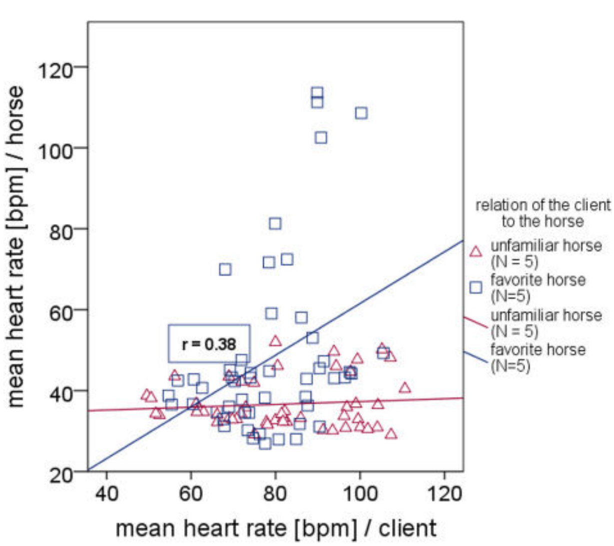

Fig. 5. Mean heart rates of clients *and horses, grouped after the relation of the client to the horse.*

3.3.2. *Synchronization between therapist and client*

The results showed no significant correlation in the HRV parameters or in cortisol between the therapist and the client. However, there was a significant correlation between the HR of the therapist and the client during the experimental condition ($r = 0.24$, $p = 0.018$) as well as during the control condition ($r = 0.31$, $p = 0.002$).

3.3.3. *Synchronization between therapist and horse*

The results indicated no significant correlation in the HRV parameters and in cortisol concentrations between the therapist and the horses. However, there was a significant correlation for HR between the therapist and the horse ($r = 0.53$, $p < 0.000$). The effect of familiarity and the relationship between the therapist and the horses was also investigated. The correlation between the HRs of therapist and the horse was stronger when the therapist was working with a familiar horse ($r = 0.61$, $p = 0.000$) compared to a less familiar horse ($r = 0.55$, $p = 0.000$).

4. Discussion

4.1. *Synchronization in EAT*

This study aimed to investigate all three participants in the triad of EAT and analyse synchronization processes. We found a significant positive correlation between the mean HR of the therapist and the clients, which was even more pronounced in the control condition compared to the experimental condition. This suggests that synchronization between humans may be facilitated in the dyadic scenario. The mean HR of the therapist also correlated with the horse, with an even stronger correlation when the therapist worked with a familiar horse compared to an unfamiliar one. The mean HR of the clients only significantly correlated with the horse when they interacted with their favorite horse, but not when they interacted with an unfamiliar horse. These results suggest that the relationship is an important modulating factor for the synchronization of HR between horses, clients, and the therapist.

Accordingly, our data complement earlier studies investigating synchronization between humans and animals. Current literature indicates synchronization of behavioral states and affects [44], converged heartbeats of mothers and their infants [82], and synchronized heartbeats of choristers [83], as well as the assumption of cross-species synchronization [41,42]. Significant correlations in human and horse HRs during EAT sessions appear to result from external stimuli [29], and horses have been shown to adapt to human HR in a fear-inducing situation [84]. Some authors have demonstrated a reciprocal influence of human and horse HR [65,70], while others could not confirm the hypothesis that the rider's stress response directly influences the horse's HR [68]. Further studies have observed both common increases and decreases in patient and horse HR, as well as differential responses [62].

In addition, literature also shows an increased synchronization through relationships. There were decreased effects on blood pressure and HR through human-animal interaction, which were more pronounced with one's own pet and seem therefore dependent on the quality of the individual human-animal relationship [85]. There was an effect of relationship and attachment style of at-risk adolescents on stress behavior and HR in therapy horses [54]. Our data also highlight the importance of relationship in the therapeutic process [86–89]. The factor of relationship seems not only important from a human point of view but also for the involved horses. With a profound human-horse relationship, horses showed significantly lower HR and less stress [90]. Compared to an unfamiliar handler, horses showed a decrease in the stress response in the HRV when interacting with a familiar horse handler. The type of interaction was also decisive for the change in the horses' HRV [91]. The importance of relationship for the well-being and compliance of animals shows an implication for daily practice in EAT to offer a safe and high-quality environment for clients.

So far, there are no clear conclusions on the process of synchronization of HR cross-species. A study by Hockenhull, Young, Redgate and Birke (2015) addressed these conflicting results and summarized existing literature, indicating that in some situations, the emotional state of humans is transferred to horses, and there is potential for synchronized HRs of horse-human pairs. They analyzed the relationship of HRs of horses with familiar and unfamiliar humans during interactions and showed that it is not consistent or straightforward and depends on a number of factors. While horses with a familiar handler had a lower HR, the opposite pattern was seen in humans [67].

Corroborating previous findings, our results indicate that cross-species synchronisation of heartbeats occurs under certain conditions. Our study outcomes identified familiarity as a modulating factor for synchrony to occur, but they also highlight the need for further research into the preconditions required for this synchronization.

4.2. Stress reduction in EAT

The other purpose of the study was to investigate potential stress-buffering effects of EAT. Young women with intellectual disabilities showed a significant lower HR in the presence of a horse compared to the control session during the challenge phase. However, during the phases of relaxation and the pre- and post-intervention baselines, no difference in HR was found.

Our results are partly in line with previous studies on animal-assisted therapy (AAT) that show a significant difference in HR after AAT compared to before the therapy [92]. Hospitalized children who received animal-assisted interventions (AAI) showed a significant decrease in HR during the course of this therapy [93] and a significantly lower HR than children who received play therapy [94]. Clients experiencing psychological distress had a lower HR when dogs were included in a modified mindfulness-based stress reduction program compared to clients experiencing the program without a dog [95]. Humans with a pet had significantly lower HR, experienced a smaller increase in HR during a stress-inducing event, and recovered faster than people without a pet [96]. After interacting with their own dog, the HR of dog owners was lower than that of the control group [97]. Some authors found a lower HR during equine-assisted interventions (EAI), but in contrast to our study, the HR decreased particularly while grooming and stroking the horse [64].

It is important to acknowledge the potential effect of walking and balancing by foot versus navigating through obstacles on horseback. Unfortunately, there was no opportunity to further control for the potential bias of movement during the challenge phase. However, between the pre-intervention baseline and relaxation phase 1, clients walked two rounds in the riding arena at their own preferred gait speed (control condition) or sat on horseback while riding (experimental condition). No confounding effect of walking by foot was seen on HR at the beginning (or during) the relaxation phase 1, that directly followed either walking or riding two rounds.

We found no effect of the presence of a horse on the clients' HRV. This contrasts with another study that found a higher HRV in participants walking with a dog compared to walking alone [98]. Additionally, a further study showed that the HR of seniors at an assisted living residence increased during guided interactions with a horse, but there were no significant changes in HRV. Sixteen of the 24 participants showed synchronized HRV peak frequencies during sessions [63].

There might be an explanation for not finding a decreased HR in interacting with the horse in contrast to the control condition over all phases. This can be explained by the fact that all participants of the study started with EAT during their childhood, and the horses accompanied them through the transition into adulthood. The surrounding of the horse stable already had a strongly positive association, which could have impacted the control condition without the horse. For each client, the location of the EAT center is linked with many positive memories and experiences. Thus, just attending the familiar stable and EAT environment might have impacted the physiological responses. The open stable of the horses is immediately adjacent to the hall. Therefore, clients could still see horses from afar even under the control condition on the barrel horse. This limitation should be controlled for in future studies. Moreover, further research should introduce a second control condition with HR and HRV measurement at a neutral location other than the familiar EAT center. Although reductions of cortisol in EAT recipients from pre-to post EAT session [99] were previously reported, our data did not reveal any significant effects of EAT on salivary cortisol secretion. It might be interesting to compare clients with rich experiences in EAT to a group of individuals who are new to EAT to parallel the effect of novelty in cortisol reactivity.

4.3. Limitations, strengths and future research

The interpretation of the results is limited by the small sample sizes of the participating humans and horses. According to a power analysis with G*Power [101], a sample size of 45 human clients would have been desirable. However, due to the limited time, financial, and personnel resources of the EAT center, the study team decided to conduct a pilot study to inform a larger-scale follow-up project. Additionally, ethical considerations made it impossible to close the EAT center, which was blocked for this research project for two weeks, for a longer period and to deny clients their regular therapy for an extended time. Due to these limitations, the results are explorative and preliminary. To draw any further conclusions, consecutive studies are required. However, outcomes from this study can be used for planning a more robust and larger-scale study.

Another aspect to consider is that due to the nature of EAT, clients cannot be blinded, as it was obvious to them if the horse was present or not.

The study took place in February during winter, which may have influenced the physiology of the clients, the therapist, and the horses. Notably, it was possible to avoid disturbances such as noise from other clients or horses, as the EAT center's activities are closed during this period of the year. The limitations of the study also include the potentially distorting effects due to the high level of standardization. The presence of a computer and an observer noting the times of data recording created an unfamiliar atmosphere, which may have intimidated the clients. Consequently, they may have interacted less with their horse or the therapist compared to the protected therapeutic atmosphere they are accustomed to.

This is the first study to include measurements of the therapist, the horse, and the client in EAT. Our results showing high levels of synchronization between the horse's and therapist's heartbeats support the previous call of the One Health framework [72], which highlights the importance of addressing all actors of the therapeutic triad in AAI. Additionally, the clients' extensive EAT experiences underline the implications for practitioners, as most studies on AAT involve only participants who have just started therapy or have little to no previous experience with it. Certainly, adding subjective measures of mental state, emotionality, and arousal would provide valuable additional insights. With a cohort like the participants from this study, such scales need to be adjusted and appropriate for the use in individuals with cognitive impairment.

The preliminary data from our study do not indicate at any acute concern for horse welfare related to participation in EAT. However, a holistic analysis of equine data and implications for practice has been reported elsewhere [100]. Our results underscore the need for further investigation into the effect of relationship intensity between all three agents in EAT. This aligns with previous calls from other authors to better understand the horse-human relationship and improve animal welfare [102–104]. For example, future studies should investigate synchronization in EAT sessions where not only the horse is familiar or unfamiliar, but also the therapist is familiar or unfamiliar to the client. The familiarity between the therapist and horses in this protocol was either high or low, but it would certainly be worthwhile to study synchronization in heart rate if the therapist interacted with an unknown horse, as no correlations in HRs were found for clients interacting with an unknown horse. It also would be interesting to compare the effects when clients interact with the horse from the ground versus sitting on the horses' back. Different body positions may have accounted for the differences between therapist and client in synchronization with the horse. Since relationship seems to be a crucial factor, assessing the neuropeptide oxytocin, which plays a pivotal role in social connection and bonding [93], might be an interesting parameter to include in future research. Additionally, future studies can benefit from calculating the area under the curve to provide additional insights on cortisol reactivity across repeated measurements [105].

CRediT authorship contribution statement

A. Naber: Writing – original draft, Visualization, Resources, Methodology, Investigation, Data curation, Conceptualization. **L. Kreuzer:** Investigation, Formal analysis. **R. Zink:** Resources, Project administration, Conceptualization. **E. Millesi:** Formal analysis. **R. Palme:** Formal analysis. **K. Hediger:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **L.M. Glenk:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization.

Ethics approval and consent to participate

The study protocol was approved by the ethics committee of the *University of Vienna, Austria (reference number: 00303)*. As the participants belong to a vulnerable group, they received a detailed briefing about the procedure, the voluntariness of participating, the anonymization of data, and an informed consent in easy language. With their signature (or the signature of the parents of the participants under eighteen), they gave their written informed consent to participate in the study.

For the horses, all methods and procedures used in the study 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) of the *University of Veterinary Medicine Vienna, Austria.*

Availability of data and materials

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

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Declaration competing interests

The authors declare that they have no competing interests and the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Preliminary results of this study were published as a conference paper (Naber A, Kreuzer L, Zink R, Millesi E, Palme R, Hediger K, Glenk LM. Heart rate, heart rate variability and salivary cortisol as indicators of arousal and synchrony in clients with intellectual disability, horses and therapist during equine-assisted interventions. Pet Behavior Science (2019) 7:17–23. https://doi.org/10.21071/pbs.v0i7.11801).

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