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Horses' Cardiovascular Responses to Equine-Assisted Group Therapy Sessions with Children

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Abstract: Children with psychosocial, developmental or physical impairments benefit from equine-assisted therapy (EAT) in multiple ways. However, to date, the animal perspective of such interventions has received comparatively less scientific dedication. Thus, heart rate (HR) and heart rate variability (HRV) of seven therapy horses that lived in an open stable environment and participated in therapeutic group sessions with children were monitored within 60 min prior to and within 90 min after EAT. Moreover, cardiovascular activity was compared to a control condition on a day without any EAT sessions. No significant differences in HR or HRV were found, neither before nor after EAT nor under the control condition. These findings do not give rise to any concern regarding horse welfare in the studied setting but cannot be generalized to a broader population of equines serving in EAT. Given the enormous heterogeneity in EAT, more in-depth research is warranted using behavioral and physiological indices of equine health and wellbeing.

Keywords: equine-assisted therapy; horse; welfare; stress; heart rate variability

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Academic Editor: Jan S. Suchodolski

Received: 25 January 2025
Revised: 26 February 2025
Accepted: 28 February 2025
Published: 3 March 2025

Citation: Kreuzer, L.; Naber, A.; Zink, R.; Glenk, L.M. Horses' Cardiovascular Responses to Equine-Assisted Group Therapy Sessions with Children. *Pets* **2025**, *2*, 12. https://doi.org/10.3390/pets2010012

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1. Introduction

Equine-assisted therapy (EAT) has been shown to be effective in various populations of recipients [1–5]. Especially children with psychosocial, developmental or physical impairments benefit from therapeutic interventions that are facilitated by horses [6–9]. In recent years, research into the animal perspective of EAT has significantly advanced, however, due to the heterogenic nature of EAT programs (i.e., their therapeutic goal, basic profession of therapists, number and characteristics of recipients, protocol, duration, time course of the sessions and time intervals between sessions), the generalizability of scientific outcomes is still limited. Accordingly, more studies are needed to reflect the diversity and complexity of EAT by addressing not only recipient health-related outcomes but also the impact on the animals involved.

In mammalian species, including equids, exposure to challenge stimulates an immediate activation of the sympathetic–adrenal–medullary (SAM) axis, which results in a discharge of noradrenaline along sympathetic fibers [10]. Since the secretion of noradrenaline and adrenaline directly impacts cardiovascular rhythmicity, SAM axis monitoring via non-invasive measurements is a suitable approach to capture arousal-related adaptations in animals [11]. Heart rate variability (HRV) analysis contains detailed information of the time span between two adjacent heart beats (=RR interval) and thereby mirrors the natural irregularities in heart rate patterns. Beat-to-beat variations originate from the interplay

between the parasympathetic and sympathetic branches of the autonomic nervous system (ANS) [12]. Stressful experiences trigger sympathetic fibers, which in turn impact the heart to accelerate and decrease variations in time intervals between individual heartbeats. In contrast, parasympathetic activity decelerates the heart rate (HR) and results in higher variations in beat-to-beat intervals.

The standard deviation of all RR intervals (SDRR) represents the overall variability of the time intervals between single heart beats [13]. A higher SDRR therefore suggests a more efficient interaction of sympathetic and parasympathetic autonomic functioning. The amount of parasympathetic activity is associated with the root mean square of successive differences (RMSSD) of subsequent RR intervals. In spectral HRV analysis, high frequency (HF) is regulated by the breath and vagal tone whereas low frequency (LF) is facilitated by parasympathetic as well as sympathetic function (Baumert et al., as cited in [13]). The frequency domain indicator LF/HF reflects the sympathetic and parasympathetic balance (Eckberg, as cited in [13]), while the standard deviation of the diameter of the scatterplot (SD1) considers the short-term variability of individual heart beats and parallels parasympathetic tone [14]. Despite the benefits of SAM axis activity in enabling cardiovascular adaptions in order to cope with challenges, too frequent and prolonged autonomic activation causes exhaustion [10]. In fact, chronic stress experiences have detrimental effects for health and wellbeing. Research into the animal perspective on EAT should ideally be based on non-invasive scientific methodology that only minimally disrupts the human–animal interaction process [15]. As the ANS integrates physical, mental and environmental stimulation, assessment of cardiovascular functioning has been considered a suitable approach in equine science [16,17].

Previous studies using cardiovascular activity as welfare indicators in therapy horses during human–animal interaction sequences have proposed inconsistent findings. Arrazola and Merkies [18] reported increases in therapy horses' HRs over the course of a 10-week EAT protocol which was accompanied by a decrease in socio-positive behaviors. In contrast, hippotherapy for disabled individuals did not affect equine HRs [19]. In a study comparing equine HR responses to people with and without a diagnosed post-traumatic stress disorder, higher HRs were found when horses interacted with traumatized individuals [20]. Higher equine HR was measured during the anticipatory and on horseback phase of EAT compared to performing groundwork [21]. Previous data indicated that the spectral HRV indicator LF/HF increased during the active working sequence compared to when the horses were prepared by the recipients [22]. Similarly, when horses were guided by recipients with an intellectual disability through an obstacle course including a slalom, crossing over a pole and balancing on a pedestal, their LF/HF increased compared to activities that were less demanding [23]. In free-ranging horses seasonal influences on HRV were found. The HR and RMSSD were lower during winter. An increase in activity, raining and humidity positively influenced the RMSSD whereas the ambient temperature negatively affected it. In free-ranging horses the parasympathetic activity was higher from 12 a.m. to 6 p.m. [24]. Therapy horses, in contrast, showed elevated values during the night and lower values during the day [25].

Thus, the aim of this study was to monitor the autonomous responsiveness of therapy horses related to their regular participation in EAT by assessing HR and HRV on days with (i.e., experimental condition) and without (i.e., control condition) scheduled group therapy sessions. If the EAT sessions substantially impact ANS functioning, a significant difference in the mean HR and parameters of HRV would be expected from pre- to post-EAT or would emerge between the two conditions.

2. Materials and Methods

2.1. Therapy Horses

Data were collected during regular activities of the EAT center Lichtblickhof, an organization offering animal-assisted therapy to children and adolescents dealing with grief, trauma, palliative diseases or disability. At Lichtblickhof, therapy horses live in a stable herd of 20 individuals in an appropriate environment, consisting of an open stable with unlimited access to paddocks and a riding hall nearby [23]. Access to hay, trees, water and enrichment elements was provided *ad libitum*. In sum, seven therapy horses of various breeds with a mean age of 17.7 ± 7.54 SD years and 8.8 ± 6.8 SD years of experience in EAT participated in the study (see Table 1).

Table 1. Therapy horse details: Age and experience in EAT are expressed in years. ✓indicates successful measurements in the respective condition—experimental (E) or control (C). x indicates loss of data.

Horse	Sex	Age	Breed	EAT Experience	(E) Condition	(C) Condition
1	f	9	Criollo	3	✓	x
2	f	23	Shetland pony mix	17	✓	✓
3	f	29	Shetland pony	18	✓	✓
4	m	25	Icelandic horse	15	✓	✓
5	m	8	Criollo	1	✓	x
6	m	8	Warmblood	1.5	✓	✓
7	f	11	Criollo	5	✓	x

At the time of the experiments, all animals were in good health, confirmed by veterinary screening (>2 times/year). Prior to the onset of the study, autonomic activity assessments were carried out for each participating horse. Horses underwent regular training sessions and were encouraged to communicate states of joy, arousal or when they require a break during participation in EAT-related activities via audible exhale communication [26]. All individuals were free from medications and displayed normal equine behavior.

2.2. Study Protocol

Each horse was measured once under the experimental condition (E) and under the control conditions (C). One EAT group session of 60 min served as the EAT session under the experimental condition. In the experimental condition, the HR and RR intervals were measured for one hour prior to EAT and one and a half hours after EAT, which took place between 2 p.m. and 6:15 p.m. No measurements were carried out during EAT to account for discretion during the therapeutic process. In the control condition, no EAT took place and horses remained in their herd during that equivalent time span. This schedule was chosen to interfere least with the ongoing EAT sessions at the EAT center. As indicated in Table 2, HR and RR intervals were recorded continuously 60 min before and 90 min after EAT but were divided into 30 min sequences for analysis. EAT only took place under E.

Table 2. Measurement schedule of the experimental condition (E) and the control condition (C). ✓indicates EAT performance, X indicates no EAT performance.

	−60 min	−30 min	EAT	+0 min	+30 min	+60 min
E	30 min	30 min	✓	30 min	30 min	30 min
C	30 min	30 min	X	30 min	30 min	30 min

2.3. EAT Sessions

EAT sessions were carried out with small recipient groups of two to four children who had a mean age of 9.4 (±3.3 SD) years and did not show any motor impairment but exhibited impaired cognitive or social functioning. The group (children, therapist and horse) was familiar and met regularly once a week in the EAT center. A low intensity of movement for the horse, mainly standing or slow walking gait, characterized the performed EAT units. That way, the comparability between conditions could be ensured best as these were the most common observable activity patterns when horses roamed freely in the open stable. EAT sessions included preparation of the horses, groundwork and guided rides in the surrounding area of the association, relaxation exercises for the EAT recipient or free interactions (including stroking, talking to the horse and playing) in the riding hall. At least one other therapy horse was present in the riding arena during EAT sessions. On control days, the time of measurement was matched to the respective one under the experimental condition, but the horse remained in the open stable instead of performing EAT. The order of the conditions was randomized and scheduled so that half of the horses started with the experimental and the other half with the control condition. In general, no additional EAT services, riding or training activities took place on the days of data collection.

2.4. Heart Rate Recordings

The HR and RR intervals were measured by a Polar® V800 telemetric device combined with an equine H7 heart rate sensor belt set (Polar® Electro Oy, Kempele, Finland). As the H7 belt set is a wireless device, it was deemed suitable for measurements in the open stable. The measurement device was always attached to the horse in the open stable. The winter coat of the horses demanded careful moistening prior to attachment. The H7 belt can easily be adjusted to the various sizes of horses and the horse's coat was wetted, which in addition to electrode gel ensured adequate contact for data transmission. An equine chest protector (normally used against grating) was put on top. A small sewed-on pocket at chest height stored the V800 monitor during measurements close to the sensor. The data collection (−60 min) began after a ten-minute acclimatization period prior to the measurement, which was deemed suitable from the procedures of an earlier study protocol [23]. Data were recorded continuously. After each measurement the data were transferred to a PC.

2.5. Activity Protocol: Behavioral Monitoring

All measurements were initiated in the open stable within the herd. Accounting for the fact that HR measurements are prone to artifacts of movement [27], changes in animal posture or gait need to be considered. Accordingly, during data collection the activity of the horse in the open stable was logged on a per second basis in Microsoft® Excel® 2016 MSO to identify periods of movement or activity to complement the heart rate data. Walking (as opposed to standing) was defined as a fluent movement of all legs in the same direction. Additionally, the time a horse spent eating was noted. Potential disturbances (people entering, noises) were recorded as well, irrespective of whether the horse showed any behavioral response to the stimulus. To log the activity pattern, the experimenter remained within sight with the horse but kept a distance of at least two meters and did not interact. Continuous focal sampling was used, where a single experimenter observed one horse at a time.

If the experimenter lost sight of the respective horse, it was logged and the experimenter changed place accordingly. After each 30 min sequence, the horse was approached calmly by the experimenter to check the data transmission of the measuring device. The activity protocol revealed that each horse stood for at least 20 min in each 30 min sequence. To minimize the influence of motion on the recordings, 20 min of standing were chosen for

the final analysis of HR and HRV within each predefined sequence of 30 min. Accordingly, the activity protocol of each horse was inspected and, starting from the beginning of each sequence, only periods of standing that lasted at least for 30 s were included to merge the final 20 min sample. If a disturbance was noted in the activity protocol, a time span lasting from 5 s before to 30 s after the disturbance was excluded to eliminate biased data. If a disturbance lasted noticeably longer than 30 s, the frequency and kind of disturbance during the respective 30 min sequence were registered and considered as a confounding factor. The time which a horse spent eating while standing was calculated within each 20 min sample.

2.6. Data Management and Statistics

A low battery status in the sensor unit of the measuring device led to abnormal HRs during the three control recording sessions, so these had to be excluded from the data pool. The final sample consisted of seven horses (N = 7) regarding the comparison within the experimental condition and four horses (N = 4) regarding the comparison between the conditions. For HRV analysis, the respective 20 min of standing were chosen in the Kubios HRV program (Kuopio, Finland) and merged into one sample per 30 min. A very low threshold level was chosen to detect artifacts according to van Vollenhoven et al. [28]. The percentage of replaced beats was noted for each 20 min sample. The characteristics of each merged sample (mean duration of the single periods of standing and the percentage of replaced beats by the artifact correction) were noted.

Within the experimental condition, each parameter (HR, SDRR, RMSSD, LF/HF, SD1) at each time point was tested for normal distribution by the Shapiro–Wilk test. The time points before EAT (−60 min, −30 min) were compared to the time points after EAT (+0 min, +30 min, +60 min), as well as once with each other. This was performed by an ANOVA for repeated measures or a Friedman test as a non-parametric alternative. A Bonferroni correction was applied, resulting in an alpha level of a = 0.0125. The comparison of each parameter at one time point (−60 min/−30 min/+0 min/+30 min/+60 min) between conditions was performed by a paired samples *t*-test or a Wilcoxon signed-rank test as a non-parametric alternative. The alpha level remained at a = 0.05.

Effect size measures were used between conditions (Glass' *Δ)* and for repeated measurements within the experimental condition ($d_{rm,\ pooled}$) [29] to evaluate the strength of EAT on changes in the horses' activity protocol.

3. Results

3.1. Experimental Condition

Regarding the HR and HRV assessments over the experimental condition (N = 7), one-way repeated measures ANOVA with a Greenhouse–Geisser correction (as Mauchly's test of sphericity showed that this assumption was not met) revealed no statistically significant main effect of time point on the HR of horses within the experimental condition ($F(4, 24) = 1.93$, $p = 0.177$, $\eta^2_p = 0.249$, see Figure 1).

Regarding the HRV indices, there was neither a statistically significant difference of the SDRR (Friedman test: $\chi^2(4) = 4.91$, $p = 0.296$) or RMSSD (Friedman test: $\chi^2(4) = 2.63$, $p = 0.622$) nor of the LF/HF (Friedman test: $\chi^2(4) = 6.74$, $p = 0.150$) or the SD1 (Friedman test: $\chi^2(4) = 2.62$, $p = 0.622$); see Table 3.

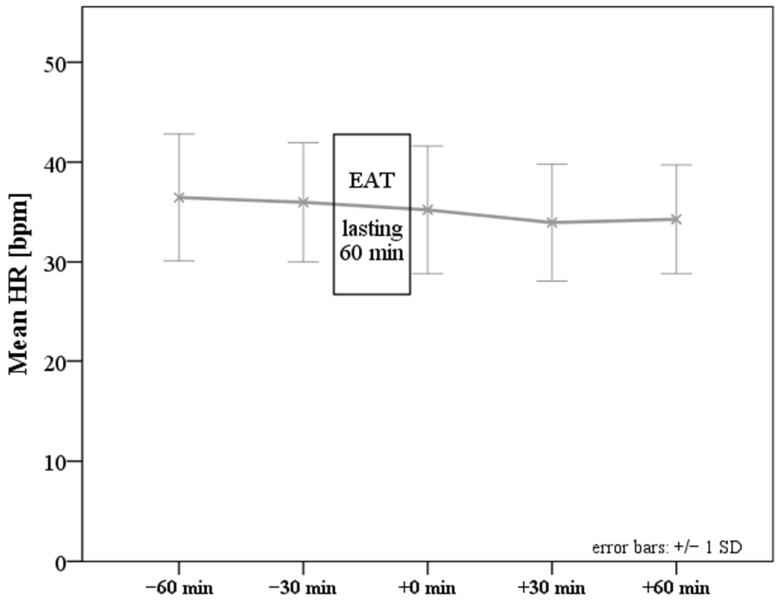

Figure 1. HR (±SD) of therapy horses (N = 7) at different time points (*x*-axis) under the experimental condition. The box indicates the time when an EAT group session took place.

Table 3. The HRV indicators SDRR, RMSSD, LF/HF and SD1 (Mn ± SD) were assessed before (−60 min; −30 min) and after (+0 min; +30 min; +60 min) EAT. The vertical lines indicate the time point of the EAT group session.

N = 7	−60 min	−30 min	+0 min	+30 min	+60 min
HR [bpm]	36.45 ± 6.35	35.97 ± 5.97	35.22 ± 6.40	33.94 ± 5.86	34.27 ± 5.46
SDRR [ms]	97.42 ± 39.96	111.72 ± 40.58	111.31 ± 61.04	110.06 ± 53.00	109.16 ± 46.38
RMSSD [ms]	115.46 ± 58.26	137.53 ± 66.10	134.25 ± 82.99	135.12 ± 70.11	130.76 ± 69.01
LF/HF	2.83 ± 0.96	2.19 ± 0.77	2.95 ± 1.33	2.57 ± 0.96	3.37 ± 1.59
SD1 [ms]	81.71 ± 41.24	97.32 ± 46.78	95.01 ± 58.74	95.62 ± 49.62	92.56 ± 48.86

3.2. Experimental Versus Control Condition

HR and HRV (Mn ± SD) at each time point were compared between the experimental and the control condition (see Table 4). The pairwise comparison of the HR between conditions by a paired samples *t*-test revealed no statistically significant difference in the measurements prior to EAT (−60 min, −30 min) or after EAT (+0 min, +30 min, +60 min). Similarly, STDRR, RMSSD and SD1 at each respective time point compared between the experimental and the control condition did not reveal any statistical significance.

The Wilcoxon signed-rank test showed no statistically significant difference in the LF/HF compared between conditions (−60 min: Z = −1.6, $p = 0.109$; −30 min: Z = −5.4, $p = 0.593$; +0 min: Z = −7.3, $p = 0.465$; +30 min: Z = 0.0, $p = 1.000$, +60 min: Z = −3.7, $p = 0.715$).

Table 4. The results of the paired samples *t*-test are shown. The intensity of the HR, SDRR, RMSSD and SD1 at different time points (−60 min; −30 min; +0 min; +30 min; +60 min) was compared between experimental condition (E) and control condition (C).

		T	df	*p*
HR [bpm]	−60 min	0.49	3	0.658
	−30 min	1.03	3	0.379
	+0 min	1.20	3	0.317
	+30 min	2.30	3	0.105
	+60 min	0.99	3	0.40

Table 4. *Cont.*

		T	df	*p*
SDRR [ms]	−60 min	1.61	3	0.205
	−30 min	−0.08	3	0.942
	+0 min	1.31	3	0.281
	+30 min	0.08	3	0.950
	+60 min	−0.89	3	0.441
RMSSD [ms]	−60 min	1.62	3	0.204
	−30 min	−2.64	3	0.078
	+0 min	0.30	3	0.784
	+30 min	−0.20	3	0.854
	+60 min	−0.78	3	0.495
SD1 [ms]	−60 min	1.62	3	0.204
	−30 min	−2.63	3	0.078
	+0 min	0.30	3	0.79
	+30 min	−0.20	3	0.854
	+60 min	−0.78	3	0.495

3.3. Behavioral Monitoring

Behavioral monitoring was carried out in order to control for artifacts of movement, activity or disturbances that could bias the HR and HRV data. The descriptive statistics (duration of the behaviors standing and eating) as well as disturbances (frequency) and the amount of replaced beats (%) for each sequence of the protocol are shown in Table 5.

Table 5. Potentially confounding characteristics of each 20 min sample at each time point (−60 min to +60 min) are listed: The mean duration of standing at a time and eating, the mean frequency of disturbances and the mean % of replaced beats by artifact correction. The vertical lines indicate the time of either an EAT session under the experimental condition (E) or an equally long time span under the control condition (C). Seven horses were included regarding the analysis within E. The comparison between conditions consisted of fewer horses (N = 4).

N = 7		$\bar{x}$	**−60 min**	**−30 min**	**+0 min**	**+30 min**	**+60 min**
E	standing	[mm:ss]	04:12	03:31	10:06	07:43	05:10
E	eating	[mm:ss]	09:02	12:00	05:25	04:05	05:14
E	disturbances	frequency	0.43	0.43	0.14	0.43	0.29
E	replaced beats	%	5.2	9.7	11.6	8.2	8.4
N = 4		$\bar{x}$					
E	standing	[mm:ss]	03:24	03:35	08:55	07:23	04:35
C			07:00	03:31	03:16	11:28	04:24
E	eating	[mm:ss]	06:30	07:11	04:49	02:09	04:39
C			12:42	07:37	11:32	02:35	02:42
E	disturbances	frequency	0.5	0.75	0.25	0.75	0
C			0.25	0.75	0.5	0	0.25
E	replaced beats	%	7.6	11.5	11.4	10.1	8.9
C			3.3	6.3	7.6	12.1	13.1

The descriptive results reveal that horses stood longer at a time and spent less time eating directly after the EAT session (+0 min) compared to before (−60 min: $d_{rm\ pooled,\ standing}$ = 0.62, $d_{rm\ pooled,\ eating}$ = −0.38; −30 min: $d_{rm\ pooled,\ standing}$ = 0.54, $d_{rm\ pooled,\ eating}$ = −0.87) within the experimental condition. The longest mean duration of standing at a time was shown under the control condition (+30 min). Compared to the experimental condition,

horses spent more time eating under the control condition at time points −60 min (Δ = 0.81; large effect) and +0 min (Δ = 0.77; intermediate effect). The frequency of disturbances was similar across both conditions. The percentage of replaced beats under the control condition was lowest at −60 min and highest at +30 min and +60 min. The percentage of replaced beats under the experimental condition was lowest at −60 min and highest at +0 min and +30 min.

4. Discussion

This study sought to elucidate the autonomous regeneration of therapy horses subsequent to EAT. Our methodological approach was centered on the request by Koolhaas et al. [30] that, in animal welfare studies, the time span of physiological recovery after confrontation with a potential stressor should be focused on. The present data revealed no statistically significant differences in HR, SDRR, RMSSD, LF/HF and SD1 between measurements before and after a group EAT session. A comparison of each parameter at each measuring time point between the experimental and the control condition did not show any statistically significant differences. The obtained results do not confirm the assumption that the service in EAT was overtaxing the internal autonomic regulation of the horses. In general, all obtained HRs remained within the range of a normal equine resting HR (25–40 bpm) as described by Evans (as cited in [11]) or Ohmura and Jones [31], thereby reflecting good health and performance [32]. Similarly, HRV monitoring revealed values within the physiological ranges described in horses under various circumstances [31,33,34]. The present findings parallel earlier EAT studies [19,25,35] but our study protocol differed from Kreuzer et al. [23], Ayala et al. [21] and Mendonça et al. [22] in that the applied measurements took place before and after EAT but not during sessions.

The absence of any SAM axis response subsequent to an EAT group session excludes a substantial influence of EAT on the ANS of therapy horses. If a stimulus has a strong effect, a time interval is identifiable before the physiological response returns to baseline values [36]. As no such interval was identifiable, the duration of autonomous regeneration, if any, was shorter than the time required to return the horses back to the stable.

In addition, no statistically significant differences were measured between the experimental and the control condition which further confirms the absence of a substantial influence of an EAT session on the ANS. In general, horses are less stressed if they either experience fewer stressors or have the ability to recover more quickly from stressors [37], suggesting that the therapy horses either did not physiologically perceive the EAT session as a stressor at all or quickly recovered from the exposure within a few minutes on the way back to their stable. Koolhaas et al. [30] proposed that, if a physiological response diminishes immediately after the stimulus exposure, the adaptive capacity of an animal is not overtaxed and the stimulus is not systemically perceived as a stressor [30]. Our previous results on therapy horses working under similar conditions are in line with the present findings, suggesting that EAT does not significantly impact autonomic and neuroendocrine cascades in equines [23]. However, the present results apply only for the performance in one group session and cannot be generalized to more frequent or prolonged EAT schedules. Thus, more frequent, longer-lasting sessions or more intense human–horse interactions could provoke relevant changes in the SAM axis response in the short term. It is likely that the frequent pre-exposure to therapeutic work in therapy horses led to a higher predictability and controllability of the situation [38], thereby positively shaping the perception of an EAT session. A recent study has proposed that equine-friendly working modalities (i.e., group outdoor housing, unlimited access to roughage, groundwork-based activities), similar to the EAT protocol of this study, may even positively impact the welfare of the horses and result in more compliance during human–animal interaction [39].

The strength of this study lies in its practical relevance. The horses were monitored during their daily routine and studied in a realistic EAT setting. As studying an animal in most cases affects to some extent what is studied [40] (pp. 125–127), it cannot be ruled out that the attaching of the measurement equipment or observation of their activity pattern did influence the horses and their physiological response. The period of acclimatization to the measuring devices (ten minutes) was deemed appropriate. The data collection at +0 min started as soon as possible to avoid the loss of important data after EAT. As no difference in the parameters at +0 min occurred compared to earlier measurements or across conditions, adjusting the measurement devices did not bias the data. In general, measuring an animal in a familiar environment reduces influences due to an artificial, unknown environment [41] but also comes with some drawbacks. The influence of people entering the open stable, herd dynamics or environmental conditions on the results cannot be exactly determined. Waiblinger et al. [41] raise awareness of the potential influence of herd mates on the behavior of the tested individual. To counteract this, such incidences were noted at the time and excluded from the analysis as much as possible. As the horses could move freely within the open stable, the experimenter had to change place accordingly to remain within range of vision to the horse. By protocolling the activity patterns, it was feasible to conduct the measurements within the open stable and no restriction of the horses, which likely would have negatively impacted their wellbeing, was necessary. Generally, it is challenging to schedule appropriate conditions to study HRV in animals because measurements can be confounded by restriction of movement but also movement itself creates a background noise [42]. To account for these difficulties, the horses were measured in the open stable and free to move and only periods of standing were included in the analysis. The measurement of free-moving horses was already successfully conducted in a study by van Vollenhoven et al. [28], who obtained more reliable HRV measurements on pasture than on stock. As the horses in our study had access to hay or trees ad libitum, eating as a potential influencing factor could not be excluded, but the duration was noted. To account for these factors, the duration of eating, the frequency of potential disturbances and the mean length of standing periods at a time (which were ultimately merged into one 20 min sample) were considered. Our data show that horses stood more at a time and spent a shorter time eating directly after EAT compared to measurements taken before. However, as horses stood longer at a time under the control condition and spent less time eating at other times, the time point rather than the intensity of the observed behaviors differed between conditions. The frequency of disturbances was within a similar range across both conditions. These disturbances consisted of people cleaning the stables and outside area as well as preparation procedures or the conduction of other EAT services in the riding hall. Such incidents occur on a regular basis in the daily routine of the horses and did not provoke an obvious response. Ideally, follow-up studies should focus on video analyses and calculate intra-rater reliability to control for the consistency in behavioral data scoring. For the control condition, future research might consider an equivalent time span where horses are moved to the riding arena and back to the open stable, similarly to the experimental condition, but without any contact with clients. This would enhance the comparability between the conditions.

To reduce the influence of the circadian rhythm, measurements were only scheduled for the second half of the day. Although it was ensured that the horses did not perform EAT or balanced training on the days of data collection, sessions on preceding days were not standardized for the purpose of the study. In this study, RR intervals were corrected by a very low threshold artifact correction. The applied method followed recommendations by van Vollenhoven et al. [28], who advised to not use a very strong correction factor and, more importantly, to report which one was applied. In fact, van Vollenhoven et al. [28]

showed that all remaining correction factors led to a similar pattern of HRV. In addition, the applied autoregressive model seems suitable for the analysis of heart beats as it estimates the spectral distribution of intervals between biological signals [43]. It has already been applied to measurements in both humans [43] and horses [11].

In general, the accuracy of the HRV measurement and analysis method influences the outcome. Appropriate artifact correction to parallel physiological variance but at the same time remove background noise and artifacts is an ongoing concern in HRV analysis. Unfortunately, a low battery status of the measuring device led to unreliable recordings with artifacts during three control measurements, which ultimately had to be excluded from further analysis. Loss of data is not uncommon in HRV field studies due to the vulnerability of the method to artifacts [22,25]. For future studies, monitoring of the battery status for each single measurement would be desirable to prevent data loss. Another limitation is that we did not assess the ANS during EAT, however, this aspect was addressed in a previous study [23].

In conclusion, our data suggest no substantial influence of EAT on the autonomic regulation pre- and post-session in therapy horses as no significant differences in HR and HRV were found. The cardiovascular welfare indicators under the control condition, where no EAT took place on that day, were similar to the measurements assessed before and after EAT. In general, EAT group sessions with children in the current study seemed of low intensity (mentally and physically) for the horses involved. Given the heterogeneity in EAT, more in-depth research is warranted using behavioral and physiological indices of equine health and wellbeing.

Author Contributions: Conceptualization, L.K., A.N., R.Z. and L.M.G.; methodology, L.K., R.Z. and L.M.G.; software, L.K.; validation, L.K., A.N., R.Z. and L.M.G.; formal analysis, L.K.; investigation, L.K.; resources, A.N. and R.Z.; data curation, L.K. and L.M.G.; writing—original draft preparation, L.M.G.; writing—review and editing, L.K. and L.M.G.; visualization, L.K.; supervision, L.M.G.; project administration, A.N. and R.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: 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 (protocol codes ETK-05/01/2018 and ETK-11/09/2018, date of approval: 17 October 2018).

Informed Consent Statement: Informed consent was obtained from the owners of the horses who were involved in the study.

Data Availability Statement: Data will be made available upon reasonable request.

Acknowledgments: We acknowledge any support that we received from the stakeholders of the organization *Lichtblickhof* to make this research project possible.

Conflicts of Interest: This article is a revised and expanded version of a master thesis by Lena Kreuzer. The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
EAT	Equine-assisted therapy
HRV	Heart rate variability
SAM	Sympathetic–adrenal–medullary

ANS	Autonomic nervous system
HR	Heart rate
SDRR	Standard deviation of all RR intervals
RMSSD	Root mean square of successive differences
HF	High frequency
LF	Low frequency
SD1	Standard deviation of the diameter of the scatterplot
E	Experimental condition
C	Control condition

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