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Original Research

The Effects of Equine Assisted Therapy on Plasma Cortisol and Oxytocin Concentrations and Heart Rate Variability in Horses and Measures of Symptoms of Post-Traumatic Stress Disorder in Veterans

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A B S T R A C T

With the increase in the number of horses being used in Equine-Assisted Activities and Therapies (EAAT) programs and with the increasing concern for animal welfare, it is important to understand the impact of such interventions on the stress level and quality of life for the horses involved. The purpose of the present pilot study was to test the hypothesis that participation in EAAT would acutely alter physiological markers of stress and well-being, including plasma cortisol, plasma oxytocin, and heart rate variability (HRV), in horses and that symptoms of posttraumatic stress disorder (PTSD) would be reduced after five sessions of EAAT in veterans who had previously been diagnosed with PTSD. Nine healthy geldings, of various breeds, ages 10–23 years, conditioned and experienced as therapeutic riding horses, were selected to participate in the study. Of these, seven were selected at random to wear electrocardiogram units, and all nine were used for blood sampling to measure plasma cortisol and oxytocin. Each horse was randomly assigned to partner with a veteran for five EAAT sessions, 1 hour in duration. A standing control was conducted on a later date on which horses did not participate in EAAT. Measurement after 5 days of EAAT was conducted immediately after the end of the last session on day 5 using the Brief Symptom Inventory and the PCL-5 (a 20 item self-report measure of the Diagnostic and Statistical Manual of Mental Disorders - 5 for symptoms of PTSD). Two way repeated measure analysis of variance showed no significant day by time interactions for plasma cortisol ($P = .821$) or oxytocin ($P = .861$). There was a significant day by time interaction ($P = .006$) for heart rate (HR); where on day 1, HR (bpm) was significantly lower during the interaction with the veterans. There were no significant differences in HRV variables. Posttherapy measures in PTSD symptoms in veterans were significantly reduced except for interpersonal sensitivity ($P = .08$) and phobic anxiety ($P = .17$). There was an effect of EAAT on HR which was significantly reduced on day 2 during the actual EAAT session. Equine-Assisted Activities and Therapies had no effect on respiration rate and systolic or diastolic blood pressure in veterans involved in five sessions of EAAT, lasting 60 minutes in duration over the course of 5 days. Stress levels, as demonstrated by plasma cortisol concentrations and HRV, did not change in horses involved in EAAT sessions with veterans who had been previously diagnosed with PTSD. Furthermore, the horses used in this study did not demonstrate increased levels of well-being as demonstrated by the lack of change in plasma oxytocin concentrations after EAAT sessions. Symptoms of PTSD did change significantly in the veterans who participated in this study.

Animal welfare/ethical statement: All methods and procedures used in this experiment were approved by the Rutgers University Institutional Animal Care Review Board and the Institutional Review Board of Monmouth University.

Conflict of interest statement: There were no conflicts of interest of concern when conducting this research.

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1. Introduction

Throughout history, there is literature that supports the notion that animals can be perceived as "healing". Overall, there is an increased interest in the general field of Animal Assisted Therapy (AAT). Though the field of equine-facilitated psychotherapy is still relatively new, a comprehensive body of evidence does exist in the literature, to support the use of animal-facilitated therapy, not specific to horses, but including work with horses, as an effective treatment for people with depression, anxiety, attention-deficit/hyperactivity disorder, conduct disorders, dissociative disorders, Alzheimer's disease, dementia, autism, and other chronic mental illnesses while reducing blood pressure, heart rate (HR), and levels of anxiety. It is also important to note that the use of AAT has had success in aiding in treatment of several physical diseases such as AIDS, rheumatoid arthritis, and cardiovascular disease. Patients who experienced AAT demonstrated a quicker recovery in comparison with patients who did not interact with therapeutic animals [1–6].

Equine-Assisted Activities and Therapies (EAAT) is an ever increasingly popular modality for people with mental and physical disabilities with over 800 certified member centers and 4,800 certified instructors offering these programs to help more than 66,000 children and adults (including more than 6,200 veterans and active-duty military personnel) involving horses [7].

With the increase in the number of horses being used in EAAT programs and with the increasing concern for animal welfare, it is important to understand the impact of such interventions on the stress level and quality of life for the horses involved. A sampling of horses of different breeds and those participating in different equine-related activities found that horses used in therapeutic riding programs demonstrated the highest amount of stress as indicated by plasma cortisol concentrations [8]. Human–animal interaction (HAI) has been investigated for its effects on hormonal indicators of stress such as cortisol, epinephrine, and norepinephrine [9].

The physical and psychological health challenges that participants in EAAT have may result in increased psychological or physical stress on the horses. A recent study hypothesized that therapeutic riding lessons would be more stressful than a standard lesson program with an experienced rider to the horses due to the nature of the physical and psychological challenges of the riders which could interfere with their ability to ride a horse [10].

Despite having a significant body of knowledge in the literature about the value of therapeutic riding on human health, there is limited insight into the impact that such activities have on the stress response and welfare of the horses involved.

There is an ever-increasing concern in society regarding the welfare of animals. Stressors are defined as environmental stimuli that lead to an imbalance of homeostasis. Responses to such stimuli include behavioral changes, reduced immunocompetence, and activation of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) [11,12]. Long-term activation of either neuroendocrine axes has been shown to be harmful. Whether a stressor has adverse effects or not depends not on the stressor itself (intensity, duration, frequency, and so forth) but whether or not the animal is accustomed to the stressor and can predict and control it [13]. In a study performed in show jumping horses of different experience levels, it was shown that horses with more experience, that were used to traveling to horse shows, demonstrated lower cortisol concentrations than their more inexperienced counterparts [14].

It is important to note that stress responses in the short term can help an organism to cope with its environment and challenging situations. If activated chronically, it may have detrimental effects resulting in impaired biological functions. Glucocorticoids and catecholamines are secreted by the adrenal gland shortly after the stressor which can be either physical or psychological. Glucocorticoids have been widely used in animal welfare research as an animal-based assessment of welfare. Activity of the ANS can be evaluated indirectly by heart rate variability (HRV) [15].

Cortisol is a natural glucocorticoid hormone produced by the adrenal cortex. Its secretion is stimulated by the sympathetic nervous system, and its main function is to mobilize energy to protect the homeostasis of vital biochemical processes during physical and psychological stress [14]. Chronic levels of elevated cortisol are also known to be correlated in a suppression of cellular and humoral immunities [16]. The effects of chronic stress can prove to be detrimental for therapeutic horses. Improving the way to scientifically assess the HPA response in horses involved in EAAT is essential to determine how the horses are coping with their interaction with humans involved in such activities [17].

Oxytocin is a neuropeptide consisting of nine amino acids produced by the paraventricular and supraoptic nuclei of the hypothalamus and has important roles in social behaviors, including reproduction and parenting. In addition, the literature shows that oxytocin is also responsible for both the causes and effects of positive social interactions resulting in a general feeling of mental and psychological well-being [18,19]. Oxytocin, released during forms of positive social interaction, may be responsible for buffering the sympathetic responses of the ANS, such as blood pressure and HR [20]. Oxytocin has antistress effects by reducing glucocorticoid stress hormones in humans and animals and is associated with increases in parasympathetic function [9].

Research has shown that interacting with animals stimulates the production of circulating oxytocin, which is known to have calming effects on the body and to aid in relaxation [21]. Work investigating positive human-canine interactions demonstrated that a significant decrease in blood pressure was found in both humans and dogs and that plasma oxytocin increases in both species, whereas plasma cortisol decreased in humans only [22]. An exploratory study looking at the interactions between people and dogs showed that physical interactions such as petting and stroking resulted in several hormonal and physiological responses in the dogs. It was noted that oxytocin concentrations significantly increased and HR significantly decreased in dogs that had interacted with people in comparison with dogs without human interactions [23]. Similar HAIs occur during EAAT.

Heart Rate Variability is a noninvasive measure of autonomic nervous activity and is defined by the natural variation in time between consecutive heartbeats. The most common reason for these variations is a deviation from the normal autonomic function [24], thus making HRV one of the most promising markers for increased sympathetic or reduced vagal activity [25]. The power spectra of HRV in the high frequency (HF) power is thought to reflect parasympathetic nervous function, whereas the low frequency (LF) power reflects both the sympathetic and parasympathetic nervous systems. The LF/HF ratio is considered to be an index of cardiac sympatho-vagal balance [26].

Research suggests that HRV analysis can be used to characterize psychological disorders such as depression, panic disorder, and anxiety. Heart rate variability analysis has shown that sympathetic activity increases and parasympathetic activity decreases during mental or emotional stress and that recall of anger increased sympathetic outflow to the heart, whereas feelings of appreciation decrease sympathetic and increased parasympathetic outflow [27]. Walking the dog resulted in increased HRV compared with walking alone, suggesting a relaxed state and an increase of parasympathetic activity [23].

Therefore, the purpose of the present pilot study was to test the hypothesis that participation in EAAT would acutely alter physiological markers of stress and well-being, including plasma cortisol, plasma oxytocin, and HRV, in horses experienced with participation in such activities. We also hypothesized that symptoms of posttraumatic stress disorder (PTSD) would be reduced after five sessions of EAAT in veterans who had previously been diagnosed with PTSD.

Specific objectives were as follows: (1) to measure plasma cortisol and oxytocin concentrations and HRV in horses participating in EAAT; and (2) to measure symptoms of PTSD before and after 5 days of EAAT and HR and blood pressure during EAAT in veterans previously diagnosed with PTSD.

2. Materials and Methods

All methods and procedures used in this experiment were approved by the Rutgers University Institutional Animal Care Review Board and the Institutional Review Board of Monmouth University. This was a pilot field study designed to measure and assess acute physiological changes in markers of stress and well-being in horses as a result of participation in five EAAT sessions with a licensed therapist and certified equine specialist.

The study was conducted in April 19–23, 2016 at the Monmouth County Park System's Sunnyside Equestrian Center in partnership with the 501c3 nonprofit organization, Special People United to Ride in Lincroft, NJ. A follow-up standing control (day 6) was conducted using the same horses on June 5, 2016 without EAAT interaction taking place. Sunnyside is accredited as a premier facility by the Professional Association of Therapeutic Horsemanship (PATH).

The study team consisted of a NJ-state licensed psychologist, Department of Animal Sciences faculty from the Rutgers University Equine Science Center, two veterinarians with expertise in equine exercise physiology and cardiology, a NJ-state licensed associate counselor who is also a PATH-certified therapeutic riding instructor and equine specialist in mental health and learning (ESMHL), and four other individuals, certified by PATH as ESMHL.

2.1. Animals

Nine healthy geldings, of various breeds, ages 10–23 years, conditioned and experienced as therapeutic riding horses, were selected to participate in the study (Table 1). Of these, seven were selected at random to wear the electrocardiogram (ECG) units, and all nine were used for blood sampling to measure plasma cortisol and oxytocin. The horses actively participated in a variety of EAAT sessions year-round and were accustomed to the farm and the arena before the start of the experiment. During the trial, the horses were kept in their usual management and husbandry routine. Horses were housed in individual stalls, fed commercially available hay and feed, and had unrestricted access to water. Horses only came inside to either eat or work in the lesson program. When not eating or working, horses were turned-out with their normal herd mates. Horses used in this study had been involved in the program ranging from 1 to 13 years. Horses were schooled by program volunteers 2–4 days per week. This includes being ridden and working on the ground. Horses worked in the lesson program up to 4 days per week, giving an average of 8–10 lessons per week. No horse worked more than 3 hours in any given day.

Horses were turned-out with their normal herd mates when not participating in the study. Each horse was randomly assigned to partner with a veteran for five EAAT sessions, 1 hour in duration. Equine-Assisted Activities and Therapies session design for each of the 5 days are shown in Appendix I.

Table 1
Description of horses participating in EAAT.

Number	Gender	Breed	Age
18	Gelding	Pinto/Paint	18
16	Gelding	Quarter Horse	15
10	Gelding	Welsh Cob/Standardbred cross	23
15	Gelding	Connemara cross	12
11	Gelding	Exmoor pony cross	18
12	Gelding	Haflinger	18
13	Gelding	Quarter Horse	15
14	Gelding	Paint	10
17	Gelding	Appendix Quarter Horse	15

Abbreviation: EAAT, Equine-Assisted Activities and Therapies.

2.2. Veterans

This study used seven veterans, six males and one female, ranging in ages from 31 to 68 years, all with a previous diagnosis of PTSD, recruited from Lakewood Veterans Affairs in Lakewood, NJ (Table 2). Veterans completed five EAAT sessions over the course of five consecutive days. Three of the seven participants had previous experience with EAAT.

2.3. Study Design

Blood samples (10 mL) were obtained via jugular venipuncture at rest, 30 minutes before (T-30) and immediately before (T0) the start of each session, 10 minutes after (T+10) and at 30 minutes after (T+30) the conclusion of the EAAT session in the horses' individual stalls to measure plasma cortisol and oxytocin. To account for the diurnal rhythm fluctuations of cortisol, samples were taken at approximately the same times each day (from 1300 to 1500 hours). Two horses were eliminated from the venipuncture procedure due to adverse reactions to the blood sampling itself. Heparinized tubes were used for plasma cortisol samples and ethylenediamine tetraacetic acid tubes were used for oxytocin samples. Samples were kept on ice until centrifuged within 1 hour of collection at 1600 $\times$g for 15 minutes at 4°C. Samples were divided into three aliquots and frozen at -80°C.

Seven horses, chosen randomly from the nine horses in the study, were used throughout for ECG data collection on three of the six study days, including day 3 as the standing control. Electrocardiogram recording was started when the monitors were placed on each horse 1 hour before the therapy sessions began, continued during the entire 1 hour EAAT session, and for an additional 1 hour after EAAT, for a total of 3 hours. For each horse, we chose to include 10 minutes of ECG data before each of the blood draws, described below, and for 10 minutes at the midpoint of the time spent with the veterans, for a total of five time periods. Blood sampling and ECG recording time courses are shown in Fig. 1.

2.4. Plasma Analysis for Cortisol

Cortisol concentrations were determined using Immunlite, a solid-phase cheminescent immunoassay system manufactured by Diagnostic Products Corporation, Los Angeles, CA, which had previously been validated for use in horses [28]. Cortisol intraassay precision was 4.8% for 3 different pools (concentrations 3.80, 4.41, and 5.67). The interassay precision for two pools of equine specimens was 10.7% and 6.7% for 28 runs at concentrations of 4.98 and 9.65. Analytical sensitivity was 0.20 μg/dL.

Table 2
Descriptions of veterans participating in EAAT.

Number	10	20	30	40	50	60	70
Gender	Female	Male	Male	Male	Male	Male	Male
Age	31	52	63	59	67	68	66
Race	E/A	E/A	E/A	E/A	E/A	E/A	E/A
Military branch	Army	Air force	Marines	Marines	Navy	Army	Army
Military years served	8	23	4	2	4	2	2
Years deployed in combat	3	2	2	1	1	1	1
Years treated at Veterans Service Organization	1	4	2	2	0	0	25
Years treated at other Veterans Affairs site	1	2	5	1	3	2	1
Combat operations	OIF	OIF/OEF	VN[a]	VN[a]	VN[a]	VN[a]	VN[a]
Prior equine therapy exposure	Yes	Yes	Yes	No	No	No	No

Abbreviations: EAAT, Equine-Assisted Activities and Therapies; E/A, European American; OIF, Operation Iraqi Freedom; OEF, Operation Enduring Freedom.
[a] Vietnam War.

2.5. Plasma Enzyme Immunoassay of Oxytocin

The measure of plasma oxytocin via oxytocin immunoassays has been validated in smaller mammals such as rodents [29]. The same validation methods, looking at parallelism, accuracy, and precision were performed in this study.

Frozen (−80°C) plasma samples were thawed at room temperature immediately before assay. Plasma Oxytocin was measured using an Enzyme Immunoassay (EIA) purchased from Enzo Life Sciences, Inc (Farmingdale, NY). The assay was performed according to the manufacturer's instructions.

Plasma was diluted in assay buffer (1:4) to give results reliably within the linear portion of the standard curve. The EIA is reported by manufacturer to be highly sensitive (minimal detection rate = 15.6 pg/mL OT) with very little antibody cross-reactivity for other neuropeptides. The chemical validity of this EIA has been assessed using tests of parallelism, accuracy, and precision. For each assay, precision was assessed by the variability between high and low controls and intraassay coefficient of variation, within known standards. Tests of accuracy resulted in a high correlation between expected and observed values. All samples were run at once and the interassay and intraassay coefficients of variation were less than 3.5%. Validation of this assay is described elsewhere [30].

2.6. Heart Rate Variability Analysis

Electrocardiogram telemetric HR monitors designed for animals (Televet 100, Engel Engineering Service GmbH, Offenbach am Main, Germany) were used 3 out of 5 days (including the standing control). Each ECG was analyzed to compute R-R intervals using the Televet 100 software, visually corrected where appropriate. Corrected R-R intervals corresponding to the five time periods were selected for HRV analysis. Heart rate variability analysis assumes that: (1) the ECG is collected during a quasi-steady state condition; (2) there are limited arrhythmias in the ECG to be analyzed; and (3) the ECGs to be compared have approximately the same number of heartbeats. The five time periods chosen met these criteria for all horses except one. The data from this individual horse were eliminated from HRV analysis because of significant second-degree heart block. Corrected R-R intervals for each time-period were analyzed for HRV using commercial software (Kubios HRV, University of Eastern Finland, Kuopio, Finland). Time-domain variables: HR, R-R intervals, and standard deviation of normal to normal R-R intervals (SDRR), and frequency domain variables: normalized LF power, normalized HF power, and LF/HF. Electrocardiogram signals were collected at 500 Hz and used for computation of HR, R-R intervals, and SDRR. Frequency domain power spectrum was computed using Fast Fourier Transform (FFT), interpolated using 5 Hz cubic spline prior to FFT, based on the Welch periodograpm method. Frequency bands for LF was 0.01–0.07 Hz and for HF was 0.07–0.6 Hz, with 512 points/Hz used for FFT.

2.7. Measurement of Symptoms of PTSD and Physiological Measurements in Veterans

Measurement of symptoms of PTSD and overall psychological distress was conducted prior to the first EAAT session on day 1 and after five days of EAAT immediately after the end of the last session on day five using the Brief Symptom Inventory (psychological distress) and the PCL-5 checklist (a 20 item self-report measure of the Diagnostic and Statistical Manual of Mental Disorders-5 for symptoms of PTSD) [31]. The Brief Symptom Inventory is a measure of self-reported psychological distress from which nine subscales are derived (including Somatization, Obsessive-Compulsive, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation and Psychoticism). There is also a composite

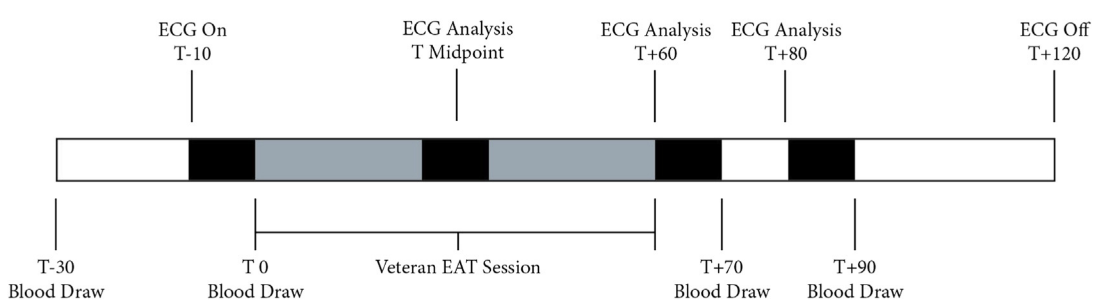

Fig. 1. Electrocardiogram telemetric heart rate monitors designed for horses were used 3 out of 5 days (including the standing control), on seven of the horses, chosen at random. Monitors were placed on each horse 1 hour before the therapy sessions began, and recording was conducted during the entire hour EAAT session and for an additional hour post-EAAT, for a total of 3 hours. We chose to analyze 10 minutes of ECG data before each of the blood draws and for 10 minutes at the midpoint of the time spent with the veterans. One horse was eliminated from the analysis because of second degree heart block. EAT, Equine Assisted Therapy; EAAT, Equine-Assisted Activities and Therapies; ECG, electrocardiogram.

subscale labeled the General Severity Index, which gives a composite or overall measure of psychological distress. The BSI consists of 53 items with symptom response alternatives: "Not at all", "A little bit", "Moderately", "Quite a bit", and "Extremely" [32].

The PCL-5 is a 20-item self-administered assessment designed to measure symptoms of PSTD. Each item addresses a different PTSD symptom as described in the Diagnostic and Statistical Manual of Mental Disorders-5 [33]. In addition to a global or total PTSD measure the PCL-5 yields four subscales which correspond to symptom clusters described in the Diagnostic and Statistical Manual of Mental Disorders-5. Cluster B Intrusive Symptoms, Cluster C Avoidance Symptoms, Cluster D Negative Alterations in Cognition/Mood and Cluster E Hyperarousal Symptoms. Chronbach alpha for the overall PCL-5 score was .95 [33].

Heart rate, respiration rate, and systolic and diastolic blood pressure were measured by two registered nurses before, during and after each EAAT session on each of the five days of the research trial.

2.8. Statistical Analysis

Data were analyzed for main effects using a two-way ANOVA for repeated measures to evaluate the differences between day and time in response to EAAT for physiological parameters, plasma cortisol and oxytocin concentrations, and time-domain variables HR, R-R, SDRR, LF/HF in the horses, and for physiological parameters, HR, respiration rate, systolic and diastolic blood pressure in the veterans. Pre- and post-PTSD and psychological distress symptoms were analyzed by paired two-tailed Student's t-test (SigmaPlot 12.5, Systat Software, San Jose, CA). Outliers were identified via Grubb's test and were excluded from statistical analysis when $P < .001$. Post hoc comparisons of means were conducted using Student-Newman-Keuls for pair wise multiple comparisons, where appropriate.

3. Results

3.1. Plasma Cortisol

Results for mean plasma cortisol concentrations for the 6 days of the study are displayed in Fig. 2. Plasma cortisol concentrations ranged from 1.54 ± 0.13 ng/dL on day 6 at T30 to 6.5 ± 0.74 ng/mL at T0 on day 3. Two-way repeated measure-ANOVA showed no significant day by time interactions ($P = .821$).

3.2. Plasma Oxytocin

Results for mean plasma oxytocin concentrations for the six days of the study are displayed in Fig. 3. Plasma oxytocin concentrations ranged from 948.7 ± 185.72 pg/mL at T+30 on day 5 to 1229.51 ± 76.2 pg/mL at T-30 on day 1. Two-way repeated measure-ANOVA showed no significant day by time interactions ($P = .861$).

3.3. HR and HRV Variables

There was a significant day by time interaction ($P = .006$) for HR (Fig. 4A); where on day 1 HR (bpm) was significantly lower at time with veterans (TV), 35.17 ± 2.22 when compared with T0, T+10, and T+30; 38.22 ± 1.46, 38.52 ± 1.81, and 37.85 ± 1.56, respectively. On day 2, HR at TV (33.90 ± 1.64) was significantly lower than at T0 (37.45 ± 1.28) but not different from T+10 or T+30, 36.06 ± 1.74 and 35.09 ± 1.59, respectively. On day 3, mean HR did not differ between time points for that day; nor did they differ from time points on days 1 and 3.

There was no day by time interaction for the standard deviation of the mean time between successive heartbeats (Fig. 4B; $P = .93$). The standard deviation of the mean time between successive heartbeats ranged from 134.15 ± 17.42 ms at T-30 on day 1 to 191.48 ± 10.15 ms at T-30 on day 2.

Regarding the LF/HF ratio (Fig. 4C), there was no day by time interaction ($P = .768$). Low frequency/high frequency rations ranged from 2.04 ± 0.24 at T+30 on day 1 to 6.63 ± 3.37 at T0 on day 2.

3.4. Symptoms of PTSD

Displayed in Table 3 are the results of differences in reported symptoms of PTSD and overall psychological distress after 5 days of EAAT. Posttherapy measures in the psychological distress symptoms as measured by the Brief Symptom Inventory were significantly reduced except for interpersonal sensitivity ($P = .08$) and phobic anxiety ($P = .17$). Also, the overall composite score of the PCL-5 indicated significant reductions in PTSD symptoms while an

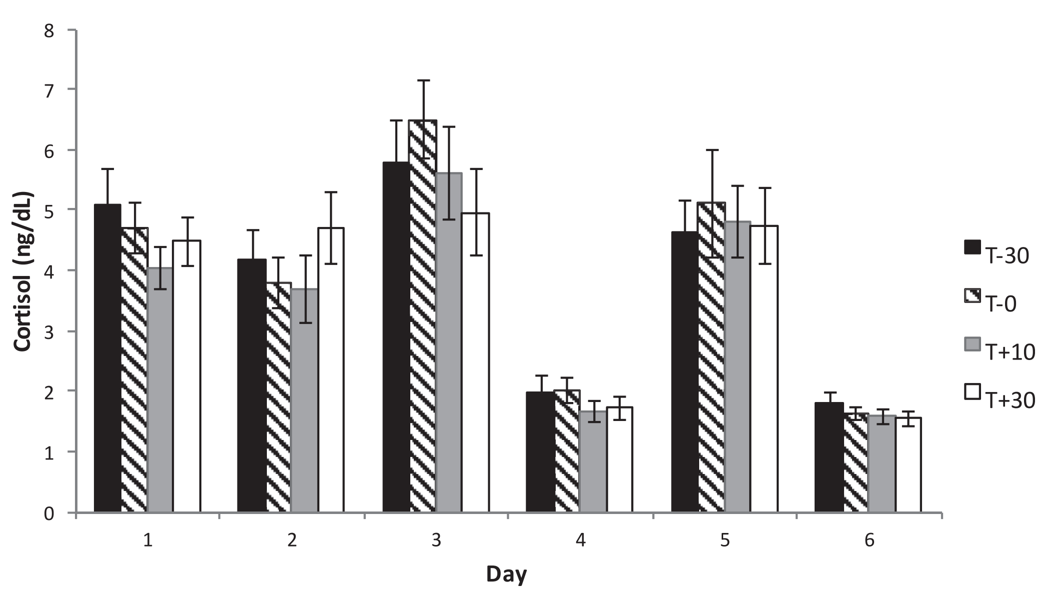

Fig. 2. Plasma cortisol concentrations (ng/dL) in horses at time -30 and 0 (pre) and at time +10 and +30 minutes after EAAT (mean ± standard error of the mean). No significant differences ($P > .05$). EAAT, Equine-Assisted Activities and Therapies.

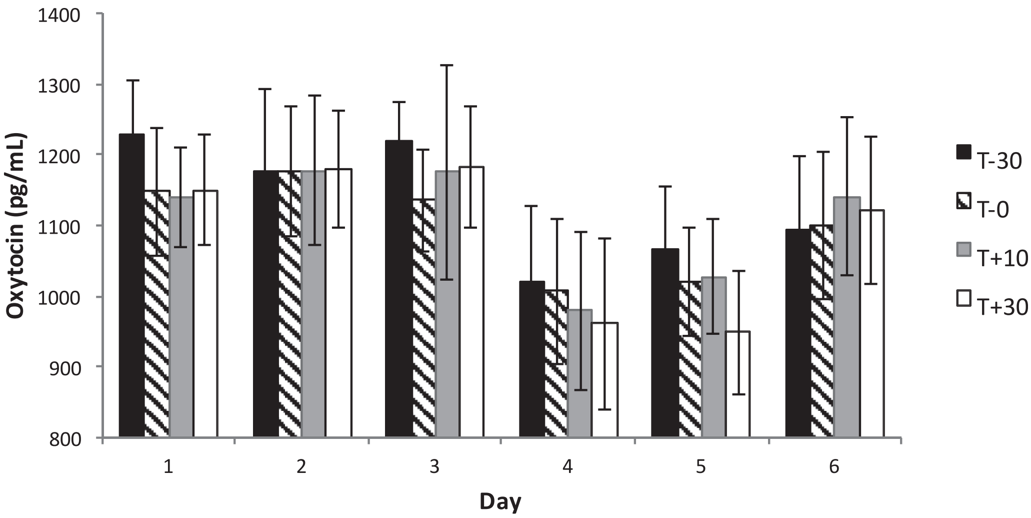

Fig. 3. Plasma oxytocin concentrations (pg/mL) in horses at time -30 and 0 (pre) and at time +10 and +30 minutes after EAAT (mean ± standard error of the mean). No significant differences ($P > .05$). EAAT, Equine-Assisted Activities and Therapies.

examination of symptom clusters revealed significant reductions in Cluster E Hyperarousal Symptoms.

3.5. Physiological Measurements in Veterans

There was an effect of EAAT on HR, which was significantly reduced on day 2 during the actual EAAT session (Table 4). This possibly could have been due to the type of activity conducted on that day which involved mostly grooming and petting the horses, in contrast to more physical activity such as leading the horse through an obstacle course. Equine-Assisted Activities and Therapies had no effect on respiration rate and systolic or diastolic blood pressure in veterans involved in five sessions of EAAT lasting 60 minutes in duration over the course of 5 days (Table 5).

4. Discussion

While EAAT have been shown to have a positive effect on people with assorted physical and psychological disorders, there is limited research reporting the effect of EAAT on the horses

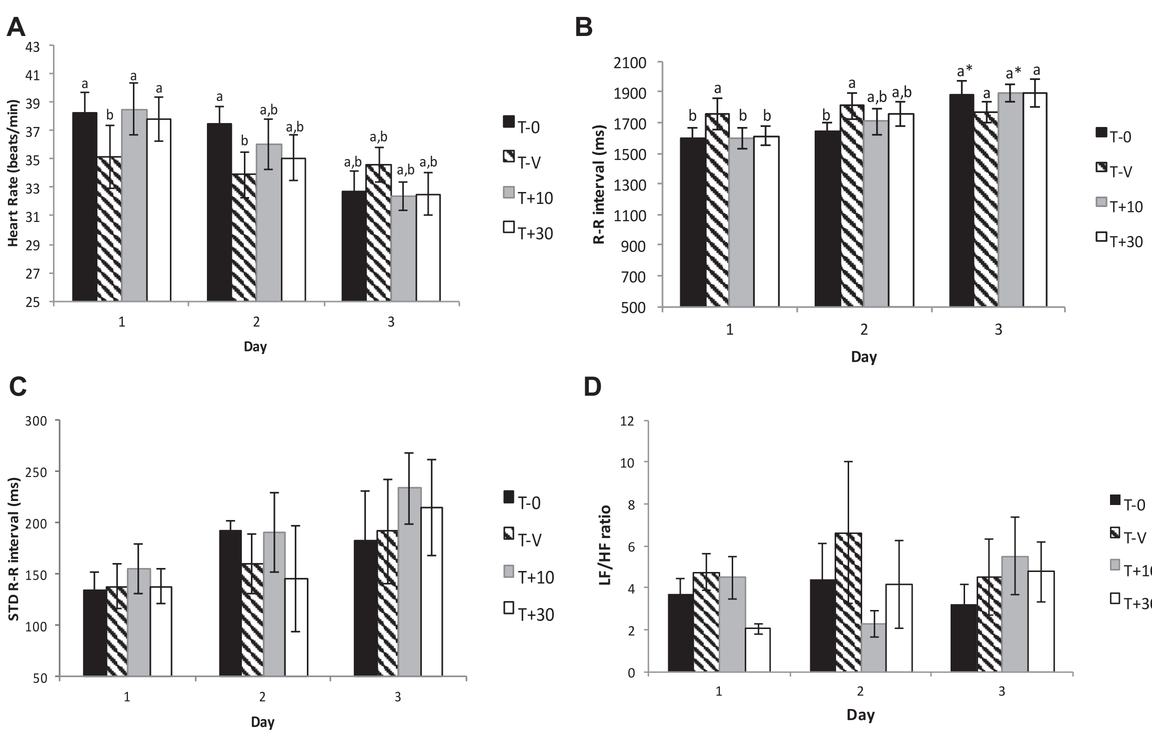

Fig. 4. (A) Heart rate (bpm) and HRV variables (B) R-R (ms), (C) standard deviation R-R (ms), and (D) LF/HF in horses at time 0 (pre), at midpoint of TV and at time +10 and +30 minutes after EAAT (mean ± standard error of the mean). Different superscripts denote differences between give time points on different days ($P < .05$). Asterisks denote differences between days at a given time point ($P < .05$). HRV, heart rate variability; LF, low frequency; HF, high frequency; TV, time with veterans; EAAT, Equine-Assisted Activities and Therapies.

Table 3
Differences in reported symptoms of psychological distress and PTSD after 5 days of EAAT.

Variable	Pre-Mean	Pre-Standard Error	Post-Mean	Post-Standard Error	*P*-Value
Brief Symptom Inventory (BSI) (Global Severity Index)	65.429	2.698	54.143[a]	3.247	.00298
Somatization	62.143	2.613	52.571[a]	2.635	.00473
Obsessive compulsive disorder	67.429	3.323	56.286[a]	2.801	.00951
Interpersonal sensitivity	57.857	4.279	48.429	5.49	.0795
Depression	53.571	2.136	48.286[a]	2.044	.0223
Anxiety	61.4229	3.993	52.429[a]	2.662	.0206
Hostility	64.286	1.569	57.429[a]	1.962	.0233
Phobic anxiety	63.714	2.447	58.857	4.114	.169
Paranoid ideation	64.286	2.834	54.714[a]	3.759	.000473
Psychoticism	62.286	4.028	51[a]	3.848	.00386
Composite Post Traumatic Stress Disorder Score (PCL-5)	59.429	3.878	48.587[a]	3.719	.0493
Cluster B intrusive symptoms	12.57	1.81	10.57	3.45	.270
Cluster C avoidance symptoms	6.14	1.46	4.71	1.60	.082
Cluster D negative cognition and mood	19.86	5.46	17.43	5.94	.219
Cluster E hyperarousal symptoms	20.14	2.85	16.14[a]	3.18	.037

[a] Denote significant differences between prescores and postscores.

involved. It was the goal of the research reported here to increase our body of knowledge regarding the horses' levels of stress and or well-being after interaction with humans engaged in EAAT. The specific objectives of the present study were to assess levels of stress and or well-being in horses involved in EAAT, as measured by plasma cortisol and oxytocin and by HRV; and to assess the effect of EAAT on several physiological measurements and change in symptoms of PTSD in veterans who had been diagnosed previously with PTSD. A limitation to the present study was the inability to measure the same hormone and physiological measurements for HRV as done in the horses due to logistics of this field study.

Cortisol and HRV were chosen as physiological markers of stress in this study. Glucocorticoids have been widely used in animal welfare research as an animal-based assessment of welfare. Activity of the ANS can be evaluated indirectly by HRV [11].

Regarding plasma cortisol, all horses displayed normal physiological concentrations of the hormone throughout the experiment, and one hour session of EAAT resulted in no change from baseline concentrations or from standing controls, which is in contrast to earlier work, which reported abnormally high concentrations of cortisol in horses used in a therapeutic riding program [8]. Another study hypothesized that horses used in therapeutic riding programs would exhibit greater increases in cortisol concentrations before, during, and after a therapeutic riding lesson, due to the challenges these riders face personally, when compared with a traditional hunt seat lesson with experienced riders, only to find that there was no difference in cortisol between the groups [10]. Although the stress response may be initiated after an exhausting physical challenge, most equine-related activities are not that physically demanding and therefore the stressor is more likely psychological [34]. Another study measured the stress response to equestrian sports and found that cortisol concentrations increased during the riding phase of the activity but returned to baseline within 30 minutes of completion. There was also an increase in sympathoadrenal activity, indicated by an increase in HR, which occurred in the stable area during grooming and saddling of the horses [35]. One study demonstrated lower cortisol concentrations in horses used in therapeutic riding at 5 and 30 minutes post-session indicating that the therapeutic riding session had small effects on the HPA response [36].

In the present study, EAAT resulted in a reduction in HR in horses on both session days, suggesting that EAAT resulted in reduced sympathoadrenal activity in these horses. Participation of horses in EAAT in this study did not result in activation of the hypothalamic-adrenal axis reflected by no change in plasma cortisol concentrations. A limiting factor in this study was the small number of animals and the large animal-to-animal variation.

The standard model of stress postulates the activation of both the sympathoadrenomedullary axis and the hypothalamo-pituitary-adrenocortical axis. Long-term activation of either neuroendocrine axes has been shown to be harmful [12]. Short-term activation of the sympathoadrenomedullary axis is used to monitor acute stress

Table 4
Heart and respiration rate in veterans pre-, during, and post-EAAT (mean ± standard error of the mean).

Day	Heart Rate Pre-Mean ± SEM	Heart Rate During-Mean ± SEM	Heart Rate Post-Mean ± SEM
1	83.43 ± 4.69	93.14 ± 3.75[a]	87.43 ± 3.48
2	83.71 ± 2.64	77.71 ± 4.64[b]	83.14 ± 3.52
3	80.14 ± 3.25	89.43 ± 3.77[a]	79.57 ± 2.83
4	87.86 ± 4.35	91 ± 5.05[a]	89.14 ± 4.66
5	84.86 ± 2.48	88.71 ± 3.54[a]	81.14 ± 2.22
Day	**Respiration Rate Pre-Mean ± SEM**	**Respiration Rate During-Mean ± SEM**	**Respiration Rate Post-Mean ± SEM**
1	17.29 ± 0.89	16.86 ± 1.35	16.29 ± 0.96
2	16.29 ± 0.27	17.71 ± 0.87	17.14 ± 0.81
3	16.29 ± 0.96	14.57 ± 0.54	14 ± 0.41
4	15.14 ± 1.00	15.14 ± 0.81	14.57 ± 0.68
5	15.71 ± 0.76	15.14 ± 1.00	15.14 ± 0.70

Abbreviation: EAAT, Equine-Assisted Activities and Therapies.
Different superscripts denote differences between days at a specific time point ($P < .05$).

Table 5
Systolic and diastolic blood pressure in veterans pre-, during, and post- EAAT (mean ± standard error of the mean).

Day	BP–Systolic Pre-Mean ± SEM	BP–Systolic During-Mean ± SEM	BP–Systolic Post-Mean ± SEM
1	135.14 ± 9.54	144.14 ± 9.3	133.86 ± 9.53
2	135.43 ± 9.26	146 ± 12.61	134.29 ± 9.16
3	126.86 ± 7.27	133.14 ± 6.65	129.43 ± 7.67
4	127.71 ± 8.69	129.17 ± 11.21	124.57 ± 8.99
5	124.86 ± 7.17	142.57 ± 10.2	134.86 ± 7.84
Day	**BP–Diastolic Pre-Mean ± SEM**	**BP–Diastolic During-Mean ± SEM**	**BP–Diastolic Post-Mean ± SEM**
1	79.14 ± 2.72	81.43 ± 1.28	80.57 ± 1.92
2	83.43 ± 81.14	88 ± 3.63	80 ± 4.29
3	81.14 ± 3.51	86.29 ± 3.30	80 ± 3.75
4	75.14 ± 1.42	83.71 ± 1.86	82.86 ± 1.00
5	74.29 ± 1.95	87.14 ± 4.09	80.29 ± 2.23

Abbreviations: EAAT, Equine-Assisted Activities and Therapies; BP, blood pressure.
No significant differences between time points ($P > .05$).

responses, while the impact of the parasympathetic part of the ANS in modulating the stress response is often ignored. It was postulated that reduced vagal activity (parasympathetic) would limit physiological and behavioral ability to cope with stress. Therefore, methods measuring vagal tone would provide more insight into individual stress vulnerability and magnitude of the stress response. Heart rate is the net effect of the vagus that slows it down thereby increasing the variability between consecutive beats and the sympathetic nerves that accelerate it. Hence, the variability of consecutive heart beats (HRV) measured using noninvasive ECG would quantify both the vagal and sympathetic activities [37].

The results of this study are in agreement with other published findings, which showed no difference in HRV variables in horses competing in dressage and show jumping but is in direct contrast to results found in assessing mental stress in Warmblood horses where the sympathetic branch of the ANS was activated, as presented by an increased LF component [35]. The vagal tone was reduced as expressed in a decrease of the HF component and consequently, the sympatho-vagal balance (LF/HF) was significantly elevated, reflecting the alteration of the ANS from rest to a highly stressed state [13]. This may reflect the experience level of the horses with the activities in which they were engaged.

In the present study, both the reduction in HR demonstrated in horses during time spent engaged in EAAT along with no differences in HRV variables SDNN and LF/HF ratio suggests that the therapy sessions were not perceived as being stressful to the horses. Coupled with no change in plasma cortisol, these findings suggest that in horses experienced with EAAT, the interaction with PTSD veterans did not result in a stress response in agreement with previous studies in experienced show jumping horses [14,38] and therapeutic riding horses [10,39].

To our knowledge, this is the first report of the measurement of oxytocin in horses used in EAAT programs. Oxytocin has anti-stress effects by reducing glucocorticoid stress hormones in humans and animals and is associated with increases in parasympathetic function [9]. We found no changes in plasma oxytocin concentrations in horses involved in EAAT with veterans. This lack of change possibly could have been due to the time of blood sampling which occurred at 10 and 30 minutes post-EAAT. Because the half-life of oxytocin is short, it may have cleared the bloodstream by the time our samples were taken. Oxytocin concentrations in dogs and their owners increased after 3 minutes of interaction, where the owner stroked, petted, and talked to their dogs [23], and oxytocin concentrations almost doubled in both humans and dogs after positive interaction whereas cortisol concentrations decreased in humans only [22]. Horses may not respond in the same manner as dogs after interaction with humans. Further research investigating the impact of horse–human interactions on oxytocin is warranted.

Animal-assisted interventions have been shown to improve cognitive, psychological, and social functioning, while reducing blood pressure, HR, and levels of anxiety in people. The use of horses to treat mental and emotional human health issues is increasing in popularity [6], and there is mounting evidence in the literature to suggest that exposure to animals may assist the overall response to treatment for people with mental illness [40]. The benefits of EAAT for children and adults with diagnoses including eating disorders [2] substance abuse disorders [41], anxiety, depression, and anger [42,43] have been documented. The results of the present study show a significant reduction in reported symptoms of overall psychological distress, with the exception of interpersonal sensitivity and phobic anxiety, after veterans participated in EAAT sessions for 5 consecutive days. These results are in agreement with a review of current literature which also reports a reduction in symptoms associated with PTSD in participants previously exposed to trauma who then received animal assisted interventions as part of their comprehensive psychological treatment plan [44]. It was noteworthy that while there were reductions in all symptom clusters of PTSD, only Cluster E Hyperarousal Symptoms were significantly reduced. Although there is currently no published research on the effects of EAAT for veterans with PTSD, the results of the present study are also in agreement with the findings of Earles [45], which demonstrated that EAAT was effective in reducing PTSD symptoms and anxiety in participants who had previously experienced a traumatic event.

In the present study, participants reported a significant decrease in symptoms of anxiety and depression along with other symptoms of psychological distress and PTSD. These results are in agreement with a study in which hospitalized patients diagnosed with psychotic and mood disorders demonstrated statistically significant reductions in anxiety after treatment with AAT [46]. A small pilot study demonstrated positive physiological and psychological responses in children who underwent a surgical procedure and were exposed to therapy dogs as part of their overall treatment plan [47]. The results of the present study are also consistent with the findings of another study in which residents of a long-term care facility reported improved quality of life indicators, specifically, decreased feelings of depression, following visitation with dogs as a component of their overall therapeutic treatment plan [48]. The U.S. Army Medical Department also published some very limited data which suggests AAT could benefit wounded warriors enrolled in an occupational therapy life skills program and supports the need for further research in the area of AAT as an adjunct therapy for veterans with PTSD and other traumatic injuries, including traumatic brain injury [49].

The results of the present study showed an effect of EAAT on HR, specifically on the day when the veterans were more stationary and were primarily engaged in grooming and petting the horses, versus actively leading and walking around. This result is consistent with the literature. One study demonstrated decreased HR when participants touched or talked to a dog [50]. Another study demonstrated decreased HR in hospitalized children who were exposed to pet therapy [51], and another finding showed decreased HR in people who petted their own dog [52]. One study also reported decreased blood pressure levels while participants were petting a dog [50]. This result conflicts with the results of the present study, which did not find an effect on blood pressure in veterans who participated in EAAT. One possible reason for this discrepancy could be the fact that veterans in the present study were standing and often moving around with the horses, whereas participants in the other study were likely much less mobile and may have been sitting down [50]. There was no effect on respiration rate demonstrated in the present study. Research on this topic has yet to yield any consistent findings with regard to the effect of EAAT on respiration rate of participants. One report concluded significant decreases in respiratory rate in hospitalized patients who were exposed to a pet therapy intervention, but there is no published data to our knowledge regarding the effects of equine therapy on respiratory rate in humans [52].

Overall, the results of the present study, which demonstrate improvement in some PTSD symptoms and limited HR reduction in participants, are consistent with the literature that suggests a wide range of potential physiological and psychological benefits to people who participate in animal-assisted interventions [9].

5. Conclusion

In conclusion, stress levels, as demonstrated by plasma cortisol concentrations and HRV, did not change in horses involved in EAAT sessions with veterans who had been previously diagnosed with PTSD. Furthermore, the horses used in this study did not demonstrate increased levels of well-being as demonstrated by the lack of change in plasma oxytocin concentrations after EAAT sessions. Symptoms of PTSD did change significantly in the veterans who participated in this study. Because this was a pilot study, further research should be conducted with a larger number of horses to study the impact of EAAT on horses used in these types of activities.

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Appendix I. Equine-Assisted Activities Therapy Provided

Day 1. Orientation to horses and overview of safety: Veterans actively participated in grooming, leading, and petting of horses. Therapist and equine specialists provided education to veterans about horse psychology and behavior. Therapist processed nonverbal cues and equine biofeedback with veterans.

Day 2. Obstacle course and mindfulness: Veterans were given the direction to design an obstacle course and lead horses through it while talking to horses about any challenges or goals that the obstacles may represent for the veterans. Veterans continued to actively participate in grooming, leading, and petting of horses. Therapist and equine specialist continued to provide education to veterans about horse psychology and behavior. Therapist continued to process nonverbal cues and equine biofeedback throughout session with veterans. Following the conclusion of the obstacle course activity, therapist led veterans through a conscious breathing exercise and introduced the concept of mindfulness.

Day 3. Horse chalking and active feelings exercise: Equine Specialists and therapist demonstrated and reviewed proper equine handling techniques for leading, steering, turning, and halting. Veterans were then instructed to use chalk to express feelings by drawing or writing words on horses' bodies. Final activity was for veterans to walk around arena and stop at signs with different "feelings" words written on them to tell horses related stories and process nonverbal cues, feedback, and body language with guidance of therapist. Veterans continued to actively participate in grooming, leading, and petting of horses throughout session. Therapist and equine specialist continued to provide education to veterans about horse psychology and behavior. Therapist continued to process nonverbal cues and equine biofeedback throughout session with veterans.

Day 4. Horse chalking and active feelings exercise continued: Equine specialists and therapist introduced new equine handling skill. Veterans were then instructed to use chalk to express feelings by drawing or writing words on horses' bodies to walk around arena and to continue to tell horses-related stories and process nonverbal cues, feedback, and body language with guidance of therapist. At the conclusion of the activity, veterans were guided to use sponge to wash away chalk on horses as a symbol of leaving negative feelings behind. Veterans continued to actively participate in grooming, leading, and petting of horses throughout session. Therapist and equine specialist continued to provide education to veterans about horse psychology and behavior. Therapist continued to process nonverbal cues and equine biofeedback throughout session with veterans.

Day 5. Termination: Therapist guided veterans through grounding activity with horse hair to make concrete association between any positive feelings that were felt during time with horses and horse hair, which they were able to take with them following conclusion of session. Veterans continued to actively participate in grooming, leading, and petting of horses throughout the session. Therapist and equine specialist continued to provide education to veterans about horse psychology and behavior. Therapist continued to process nonverbal cues and equine biofeedback throughout session with veterans.