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Cover Page Footnote

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Effects of Equine Interaction on Mutual Autonomic Nervous System Responses and Interoception in a Learning Program for Older Adults

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Abstract Equine-assisted learning (EAL) may improve the health of older adults, but scientific data are sparse. This study investigated whether people aged 55 and older show increased heart rate variability (HRV) during EAL and awareness of bodily sensations that are overall pleasant. Subjects (n = 24) participated in mindful grooming during which they slowed their breathing and brushed a horse while noticing sensations in their body and watching the horse's reactions. The subject's and horse's HRV were recorded simultaneously before, during, and after mindful grooming. For control, the same subjects performed mindful grooming with a plush simulation horse. During exit interviews, participants described their sensations. Words and gestures were categorized as positive, neutral, or negative. During mindful grooming, human heart rate and HRV (standard deviation of interbeat interval, SDRR) increased compared to baseline (paired t-test, $t = -4.228$, $p < 0.001$; $t = -3.814$, $p = 0.001$), as did the percent very low frequency (%VLF) component of HRV ($t = -4.274$, $p < 0.001$). Equine HRV values remained in the normal range, mostly VLF. In 10 cases, during mindful grooming, horse and human HRVs showed matching VLF frequencies. Grooming the simulation horse significantly elevated SDRR but did not alter %VLF. Exit interviews revealed significantly more positive gestures ($t = -3.814$, $p = 0.031$) and fewer negative gestures (Wilcoxon signed-rank test, Z-statistic = –2.12, $p = 0.036$, $p < 0.05$) when participants spoke about the real horse compared to the simulation. These findings demonstrate that during *mindful grooming* people aged 55 and older benefit by experiencing increased HRV, heightened awareness of pleasant bodily sensations, and often some synchronization of their HRV frequency spectrum with that of their horse, possibly reflecting emotional bonding.

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IAHAIO International Association of Human-Animal Interaction Organizations

Introduction

During equine-assisted learning (EAL) a participant interacts with a horse, under the guidance of a professionally trained facilitator, to develop life skills and achieve educational, professional, or personal goals. Horses are sensitive to subtle changes in the environment, making them excellent partners in communication and awareness. Equine-assisted learning programs are now being offered to health care providers (Walters, 2020) and to older adults in assisted living residences (Yu, 2017). The long-term aim of this study is to develop a safe, effective, easily implemented EAL activity that can be used with older people to improve their physical and emotional health.

Because of the growing interest in this field, scientific studies are important to ensure that EAL activities are performed humanely, benefit humans, and do not adversely affect the horses. In addition, by making physiological measurements on horses and humans simultaneously we can learn about the unique contribution horses bring to the relationship, including providing feedback for humans in enhancing their emotional regulation skills. Currently only a few published studies have reported simultaneous physiological measures on horses and humans as they participate in EAL (Baldwin et al., 2018, 2021; Lanata et al., 2016).

The present study is one of a set of three that was designed to prove the efficacy of EAL in the 55-plus age group in support of an equine program being offered in an assisted living community in Tucson, Arizona. This age range was chosen because in the United States, retirement communities that encourage an active lifestyle are marketed to adults aged 55 and older. This population will especially benefit from EAL if research shows it improves regulation of their autonomic nervous system (ANS) and helps build skills in emotional regulation. Older adults are prone to physiological and psychological disorders related to ANS imbalance, such as hypertension, cardiovascular disease, immune dysfunction, anxiety, depression, and low self-esteem (Baldwin et al., 2018, 2021). A standard measure of ANS regulation is heart rate variability (HRV), or how heart rate (HR) varies with time (Task Force, 1996). Both HRV and interoception have been found to predict outcomes of mental health and well-being (Pinna & Edwards, 2020). *Interoception* can be *defined* as the sense that allows one to answer the question, "How do I feel?" in any given moment. However, these two outcomes have usually been investigated independently of each other. In the present study we use both HRV and interoception to determine whether EAL improves autonomic and emotional regulation in older adults.

Heart rate variability in humans declines with age. The middle 50% of 20–25-year-olds usually fall in the 55–105 ms range, while 60–65-year-olds are normally between 25 and 45 (Moore, 2021). Increases in HRV during specific EAL activities or therapies have been reported (Baldwin et al., 2018; Ecker & Lykins, 2019; Garcia-Gomez et al., 2020; Gehrke et al., 2018), reflecting enhanced ANS regulation. However, due to methodological limitations and the variability of protocols between studies, more rigorous experiments need to be conducted. Specifically, trials should be performed with larger samples, precise descriptions of interventions, control groups, random group assignment, and blinding of participants and research personnel regarding group assignment where possible. The present study aims to fulfill these criteria as well as providing simultaneously recorded HRV data in horses and humans.

Increased HRV is associated with efficacy of immune function (Ernst, 2017), cognition (Colzato et al., 2018), and emotional balance (McCraty et al., 1995). When people experience stress, usually the sympathetic branch dominates the parasympathetic and HR and cardiac contractility increase. At the same time, parasympathetic stimulation, which decreases HR and contractility, is diminished. Due to the dominance of sympathetic activity during stress, the heart is inhibited from adapting to changing circumstances and the HR shows little variation, meaning that HRV is lower than normal. Ideally, a rhythmic interplay between the sympathetic and parasympathetic branches allows the heart to adapt to physical and emotional needs, leading to decreased stress and anxiety, improved mental and physical performance, and a more synchronized connection between the heart and the brain (McCraty & Zayas, 2014). This state is reflected by increased HRV. Higher HRV is associated with improved cardiovascular function (Soares-Miranda et al., 2014) and emotional health (McCraty et al., 1995; Porges, 2003; Segerstrom & Nes, 2007; Traina et al., 2011). Since HRV tends to decrease with age, these benefits may be lessened in older adults, stressing the importance of making behavioral and lifestyle changes that enhance HRV.

A recent review (Garcia-Gomez et al., 2020) supports using HRV to quantify the physiological effects of humans engaging with horses. In most studies human HRV increased after the interaction (i.e., Baldwin et al., 2018; Ecker & Lykins, 2019; Gehrke et al., 2018), indicating greater interplay between the sympathetic and parasympathetic ANS branches. The study by Ecker and Lykins (2019) examined short-term interactions of 9 individuals with 5 horses. Results were mixed, with HRV increasing in some cases and decreasing in others, although on average HRV increased. It was concluded that interacting with horses in a therapeutic/EAL setting can be either relaxing or arousing depending on the person. In another study, three groups of 5–7 veterans participated in an 8-week horsemanship program involving guided activities with horses, including grooming. The participants showed increased HRV and enhanced parasympathetic tone during and after each session, indicative of a relaxed, healthy state (Gehrke et al., 2018). However, there was no control condition, and once again, the number of subjects was small.

The present study focuses on the HRV response of older people to EAL, a population that has so far received little attention. Since older people may become physically and mentally fatigued by new challenges, a simple EAL activity is needed. For that reason, mindful grooming, a brief breathing exercise followed by five minutes of quietly brushing the horse while paying attention to bodily sensations, was selected. Awareness of bodily sensations provides quick access, via the insula (Gogolla, 2017), to how one is feeling in the present moment and motivates one to quickly temper one's emotional and cognitive responses if the sensations cause discomfort. Such modification can be achieved by slowing breathing, relaxing one's muscles, or engaging in rhythmic movement (Price & Hooven, 2018). Such activities influence sympathetic and parasympathetic neural inputs, and hence HRV (Chouchou et al., 2019) and may contribute to the feelings of well-being and the improvement in HRV that have been reported after EAL (Baldwin et al., 2018).

In this experiment, HRV was measured simultaneously in both horse and human to determine whether there was any physiological synchronization (shared HRV peak frequencies between horse and human) during the interactions. Apart from two previous studies by Baldwin et al. (2018, 2021), only one other investigation recorded simultaneous measurement of HRV in humans and horses engaged in EAL (Lanata et al., 2016). All three studies indicate that there is synchronization between horse and human heart rhythms under certain conditions. Other investigators have proposed that by analyzing physiological variables (brain/heart/hormonal) measured during horse-human interaction, the presence of a unique coupling system can be assessed (Scopa et al., 2019).

The neurobiological system underpinning physiological synchronization has never been investigated but oxytocin has been suggested as a possible candidate (Palmieri et al., 2021). Physiological synchronization is clinically relevant because it appears to be linked to emotional bonding, at least between humans. A study examining HRV synchronization in 10 groups found that synchronization between participant pairs was correlated with the degree of emotional bonding existing between those pairs (Morris, 2010). Bonding is also important between humans and animals because it benefits the health and well-being of both species; hence the investigation of HRV synchronization between humans and horses in the present study.

The equine HRV measures were also used to determine whether the horses remained within the normal HRV range during mindful grooming and were not being physiologically challenged. Previous research indicates that horses selected for EAL do not experience physiological stress during EAL or therapy (Janczarek et al., 2018; Malinowski et al., 2018) and appear to respond neutrally (Mendoca et al., 2019). However, it is important to test many different horses in a number of EAL situations before generalizations can be made regarding equine comfort and safety.

It was hypothesized that mindful grooming would increase the HRV of human participants and that the bodily sensations they experienced would be pleasant overall. In addition, the horses were expected to maintain normal HRV throughout the activity.

Research Questions

1. During mindful grooming, do older people experience an increase in HRV?
2. During mindful grooming, do older people become aware of bodily sensations that are overall pleasant?
3. Does HRV in horses remain within the normal range during unmounted EAL?
4. Does participation of horse-human pairs in mindful grooming cause synchronistic changes in HRV?

Materials and Methods

In this study, HRV was measured in adults aged 55 or older before, during, and after participating in mindful grooming on four consecutive days at an equine center. The same participants also performed *mindful grooming at the same location* with a simulation horse as a control arm. To test whether human responses to the simulation horse were being influenced by the presence of real horses in fields adjacent to the arena, a separate control experiment was completed on a group of additional participants who groomed a simulation horse in a university office far removed from any horses. During each activity participants were instructed to pay attention to the horse's responses and to focus on sensations they felt in their own body. After each interaction at the equine center, but not at the office, a video-recorded exit interview was conducted by the videographer, in which participants were asked to describe the feelings or sensations they experienced in their bodies. Interviews took place in the barn aisle, away from other participants. Studies at the equine center were performed in May 2015 with 24 adults at EquuSatori Center, a private, 11-acre rural setting with multiple pastures in Sebastopol, California. Twenty participants in the same age range participated in the office control arm.

The protocol met all criteria for IRB approval for research under Code of Federal Regulations 46.111 and was approved by the University of Arizona, Human Subjects Protection Institutional Review Board (IRB) (Protocol #1802244539) and the Institutional Animal Care and Use Committee (IACUC) (Protocol #15-054). Professional Association of Therapeutic Horsemanship standards and guidelines were followed in all situations. The number of subjects was chosen prior to the experiment by performing a power analysis on preliminary data comparing HRV before and after a meditation exercise used in another study in which 30 participants (aged 30–69) focused on sensations in the palms of their hands (Baldwin & Schwartz, 2012). Power was estimated assuming standard α values (95% confidence limits or $\alpha = 0.05$). The calculated power for $n = 20$ was 0.87 and was above the sufficient statistical power of 0.8. Therefore, using $n = 20$ would ensure that the data obtained were statistically meaningful. This number was chosen for the office group. Four additional people were recruited for the experiments at EquuSatori Center to account for attrition because individuals in this group had to attend for two days instead of one.

Participants

Humans. Participants were recruited by word of mouth and flyers prior to the commencement date of the study. To enroll in the study, human participants had to be aged 55 or older, able to stand and walk for 30 minutes, have no known cardiac arrhythmias, and have no metal plates, pacemakers, or similar devices in the body to prevent possible interference with heart rate monitors. Participants were selected who had not ridden or owned horses within the last five years to reduce the variation in their familiarity with horses. For the EquuSatori group there were 20 females and 4 males, all Caucasian, with a mean age of 62 (range of 55–73). For the university office group there were 17 females and 3 males, all Caucasian with a mean age of 61 (range of 50–77). None of the participants smoked and none were currently taking prescription drugs other than one who took thyroid medication. All participants were required to abstain from caffeine before testing and not eat for one hour before testing, and all signed the research consent form. In the case of the EquuSatori group, the EquuSatori Center release from liability form was completed in the office with the help of the administrator. None of the consented participants had visited the barn before or were familiar with the horses, and no interaction was allowed with the horses on site before testing.

Horses. The three horses at EquuSatori that participated in this study are part of an EAL program for continuing nursing education and so are accustomed to quietly interacting with people in ground-based activities. Having three horses in the study meant that each horse was groomed by eight people over four days, which was a usual level of human contact for them. Details of the horses were as follows:

Opalo: Andalusian gelding, 13 yrs, 16.2 hands
Prescott: Arabian gelding, 9 yrs, 15.2 hands
Sunday: Paint/Quarter horse mare, 9 yrs, 14.3 hands

The horses met standards of health and treatment for PATH International and had been identified as being particularly willing to engage. The horses were closely examined each day by team members who were EAL professionals to ensure that they were healthy and willing to engage in activities. If they had shown any agitation, they would have been dismissed. All horses were actively ridden and regularly engaged in EAL programs, providing them with a moderate degree of exercise and interactions with people, which they seemed to enjoy. The HRV sensor strapped around the girth area resembled a saddle girth and did not cause any adverse reaction.

Setting

The facility used at EquuSatori Center was an indoor equine block containing 12 stalls each with an attached 12 feet by 16 feet run, all separated from a covered dressage arena by an aisle 12 feet wide. A tack room, aligned with the stalls, was also included in the block. The tack room opened onto the aisle at the front and had a rear door opening onto an office at the back. The office also had a door to the outside. Participants would enter the office, where they would have their pre-intervention measures and then pass through the tack room into the aisle where their horse would be waiting for them. After their interaction with the horse, they would exit the aisle through another door to the outside and walk back into the office for their postintervention measure. A photo showing the alignment of the stalls, aisle, and arena is shown in Figure 1.

Figure 1. Arrangement of stalls (left), aisle (center), and arena (right).

Experimental Design

For the EquuSatori group, crossover repeated measurements design was used: that is, the participants crossed over from the experimental treatment to the control during the trial, thus minimizing the effects of person-to-person variation.

The 24 subjects were randomly divided into four groups of six by writing each participant's name on a card, manually shuffling the cards (four riffle shuffles), and assigning each consecutive card into one of four groups in turn. Each group interacted with a real horse or a simulation horse one day and the opposite the next day, either in the mornings or in the afternoons. Human participants and all three horses in the study had their HRV recorded before, during, and after the interaction. Human participants were given an exit interview after each of their two interactions.

The protocol followed by each participant on their first day is shown in Table 1a. On the second day, steps 1–3 were omitted, and participants switched to either simulation or real horse depending on their assignment the previous day. Table 1b shows the schedule with the assignment for each group on each day. The pre-intervention HRV of the horses was recorded just before the arrival of each group of participants. Heart rate variability of the horse being groomed was recorded simultaneously with that of the person grooming, and HRV of all participating horses was recorded just after the last person in a particular group completed their grooming session.

As shown in Table 1b, each group came for two consecutive half-days. Half the participants were randomly assigned to come in the morning and half in the afternoon on both days. Subjects arrived at scheduled times to avoid having to wait while others were completing their sessions. Half the subjects groomed the real horse on the first day and the simulation horse on the second day. The order was reversed for the other half. Each of the three horses was groomed by two people. Assignment of the horses to each person was random, based on selection from shuffled name cards. Heart rate variability was measured in human participants before, during, and after they mindfully groomed a real horse and

Table 1a Protocol Followed by Each Participant on Their First Day

1.	Listen to project description in office with group (10 minutes)
2.	Sign consent form in office with group
3.	Watch videos on safety and mindful grooming in office with group (15 minutes)
4.	Have baseline HRV recorded for 5 minutes in office
5.	Perform alpha breathing and mindful grooming of real horse or simulation horse in barn aisle *without other group members present* (10 minutes)
6.	Have exit interview in barn aisle (5 minutes)
7.	Have post HRV recorded for 5 minutes in office

Table 1b Schedule Indicating Group Activity on Each Morning and Afternoon

	Day 1	Day 2	Day 3	Day 4
AM	Group 1	Group 1	Group 3	Group 3
	Real Horse	Simulation	Simulation	Real horse
PM	Group 2	Group 2	Group 4	Group 4
	Simulation	Real horse	Real horse	Simulation

a simulation horse for 10 minutes each. The horses' HRV was measured before, during, and after each interaction.

For the university office group, each person came to the office at their appointed time and their HRV was measured before, during, and after they mindfully groomed a simulation horse for 10 minutes.

Simulation Horse

The control activity was grooming a tan color plush simulation horse with a short mane and tail. The simulation was four feet long and two feet high, in a lying down position with its head raised. It was placed on a round table (30 inches high) in the same place where the horses stood for grooming (or in an office remote from horses for the university office group) and at the same level as would be normal for grooming a live horse. The reason for having participants groom a plush simulation horse as a control activity was so that the shape and texture of the live horse would be retained as well as the grooming actions. The element distinguishing the two conditions was the presence of a live horse.

Detailed Procedure

Pre-Measures of Horses. The HRV of the three horses was recorded for five minutes as they stood, haltered and tied to a post, in the wide aisle within the barn and adjacent to the arena.

Participant Introduction. On the first day participants were greeted and shown into an office attached to the barn but separate from the horses. Two of the investigators (Ann Baldwin and Lisa Walters) introduced themselves, explained the research project and gave a general overview of the process. Then release and consent forms were signed. Then subjects were shown two videos, one on safety around horses and the other on mindful grooming. The safety video demonstrated how to read the horse's body language in response to their grooming, such as stomping feet, ears back, tail swishing for irritation or discomfort, and licking and chewing, ears in neutral position, eyes relaxing, and head down for pleasurable response. The mindful grooming video demonstrated the grooming process, including the "alpha breathing" they would implement prior to engaging with the horse. The descriptor "alpha" refers to the alpha brain waves produced by this breathing, indicating a state of wakeful rest (Park & Park, 2012). The university office group was just shown the video on *mindful grooming by one of the investigators (Ann Baldwin).*

Interaction Protocol. Pre-intervention HRV was recorded for 5 minutes. Then the EquuSatori subjects were escorted into the wide aisle next to the arena where the facilitator (Lisa Walters) stood with them (10 feet away from the horse) and guided them in a 2-minute "alpha breathing" exercise. The protocol for the university office group was similar, except they were guided in "alpha breathing" by Ann Baldwin. Instructions were to focus on the area around their heart and imagine their breath moving in and out of this area, inhaling to a count of four, holding to a count of four, and exhaling to a count of four. The goal was to breathe slower than normal.

At this point HRV monitors and video were started simultaneously to record the grooming activity. The investigators, students, and technicians were already trained to operate the monitors and knew how to behave around horses. After two minutes of the alpha breathing, the subject was motioned to move toward the horse and begin the mindful grooming process in silence. The horse had been haltered and tied to a post in the wide aisle adjacent to the arena. The subject stepped into the horse's space, allowing the horse to smell the back of their hand as a consistent first encounter with the horse. Then they picked up a soft brush and began grooming, not to "get the horse clean" but rather to observe feelings and sensations within themselves while paying attention to the horse's response. Participants were instructed to interact similarly with the simulation horse except for attending to the horse's responses. After a total of 10 minutes, including the 2-minute coached breathing, the subjects moved away from the horse for the postgrooming interview, which was video recorded.

The questions asked were:

1. What sensations were you aware of experiencing?
2. At what point were you aware of sensations?
3. Is there anything else you briefly want to share?

Each participant was interviewed twice, once after interacting with the real horse and once after the simulation horse. Following grooming, the subjects' HRV was recorded once more for 5 minutes in the office. The horses' HRV was also recorded for 5 minutes as they stood in the barn aisle.

Experimental Measures

Heart Rate Variability Parameters. The degree of change in HR over time is measured as (1) the standard deviation of the interbeat interval (SDRR) that reflects the size of the variation, and (2) the root mean square of successive differences (RMSSD), related to parasympathetic stimulation of the ANS. The frequency of the HRV oscillations is divided into three ranges: very low frequency (VLF: 0.003–0.04 Hz) an intrinsic rhythm of the heart associated with good health, low frequency (LF: 0.05–0.15 Hz), and high frequency (HF: 0.15–0.4 Hz) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The LF power (or %LF), or strength of the LF oscillation, reflects both sympathetic and parasympathetic activities (usually mainly sympathetic), and the HF power (or %HF) reflects parasympathetic activity. Based on our results with another closely related EAL activity, Con Su Permiso, or "with your permission" (Baldwin et al., 2018), as each human interacted with their selected horse during *mindful grooming*, we expected an increase in their HR, SDRR, and %VLF.

Analysis of Heart Rate Variability. Pulse measurements (interbeat intervals) were made on humans using a Zephyr Bioharness BT and on horses using a Polar Equine RS800CX belt around the girth, as described previously (Baldwin et al., 2018). The Zephyr Bioharness provides excellent reliability (Nazari et al., 2019), and Polar HR monitors provide statistically reliable data from horses from day to day in different environments (McDuffee et al., 2019). Analysis of the data was performed using the Kubios HRV Standard software program to obtain HRV parameters. For this program the detrending/smoothing function was off, keeping the VLF rhythms in the data. For the HRV data recorded during mindful grooming, only the last 5-minute portion was analyzed, so that the time duration matched the 5-minute pre and post recordings, and so HRV recorded during the initial alpha breathing coaching without grooming was not included. To determine whether there was coupling of HRV frequency peaks between horse-human pairs, Fast Fourier Transform analysis was performed by the Kubios software to access the HRV frequency domain. Using this analysis, the peak HRV oscillatory frequencies for the horse and human recordings could be compared.

Exit Videos

Each participant was interviewed by the videographer. Answers to the three questions listed in the "Interaction Protocol" were recorded using a Sony HDR-CX240 video camera. The goal of the exit interview was to determine whether the participants' sensory experience of mindful grooming was overall positive, neutral, or negative. The words and gestures used in exit interviews were quantitatively analyzed by two physiology undergraduate students to assess the comparative effects of equine and control mindful grooming on the participants' sensory experience as described in Baldwin et al. (2018). Briefly, the exit videos were transcribed, and any adjectives describing feelings and sensations experienced during mindful grooming were highlighted. Gestures made by the participants during the interviews were systematically recorded by the students watching the videos. Neither student had been present at the data collection events and both were trained by a lead investigator to analyze words and gestures. The analysis process was validated by having both students rate the first three exit videos for words and gestures to assess interrater reliability. The results were very similar. This calibration process has been repeated with three other pairs of students involved in three other related studies, further confirming the validity of the rating procedure

Words. Any adjectives used to describe feelings or sensations were separated into groups based on whether they had a positive, neutral, or negative connotation. These connotations were decided based on several information sources (Chi, 1997; Common Adjectives Table, 2016; Pierce, 2011). The total numbers of positive, neutral, and negative words were determined for each interview and the averages and standard deviations calculated for mindful grooming with the real horse and with the simulation horse.

Gestures. The nonverbal portion of the interview is important because the interviewee may not be able to verbally express how they felt or may not tell the full truth about what they felt. For this reason, gestures made by participants while describing their experiences were also categorized as positive, negative, and neutral. The gesture classification assembled for this study is shown in Table 2. Details about how words and gestures were classified are described in Baldwin et al. (2018).

Table 2 Classification of Gestures from Video Recordings

Positive	Neutral	Negative
Eye contact	Hands on ears	Fiddling
Lifts arms	Touches self	Clasps hands
Smiles	Touches something else	Furrows eyebrows
Palms open	Deep inhalation	Hands behind back
Rubs palms	Eyes closed	Purses lips
Moves arms	Head tilt	Shakes head
Nods	Blinks	
Laughs	Arms to side	
Widens eyes	Looks away	
Steps	Shrugs	
	Licks lips	
	Lifts eyebrows	

Statistical Analysis

The data analysts were blinded regarding the grouping of the data (experimental vs. control). Paired *t*-tests were run for HRV to test for significant differences between pre versus during and pre versus post for horse-related and control activities. A *p*-value of less than 0.05 was considered to be statistically significant. SigmaStat software was used for the analysis. The Shapiro-Wilk test was run for each comparison. Almost all distributions were normal; in the cases of non-normal distributions the Wilcoxon signed-rank test was substituted for the paired *t*-test.

Results

The results are presented in terms of how the data address each of the four questions posed in the introduction.

During mindful grooming, do older people experience an increase in HRV?

Examples of typical HRV recordings from a human participant mindfully grooming a real horse and the simulation horse are shown in Figure 2. In both cases, a wavelike pattern can be seen for the initial one or two minutes of the recording when participants are being coached in alpha breathing.

As shown in Table 3 (a and b), human HR, averaged over each recording period, increased significantly compared to baseline during interactions with horses (normal distribution, paired *t*-test, $t = -4.228$, $p < 0.001$) but not with the simulation horse. In contrast, HRV (SDRR) significantly increased compared to baseline during interactions with both horses and the simulation horse (real horse: normal distribution, paired *t*-test, $t = -3.814$, $p = 0.001$; simulation horse: normality failed, Wilcoxon signed-rank test, Z-statistic = 2.575, $p = 0.008$). As shown in Table 3(a) there were no significant differences between any of the parameters when comparing pre-intervention versus postintervention data for grooming the real horses, indicating that the changes observed

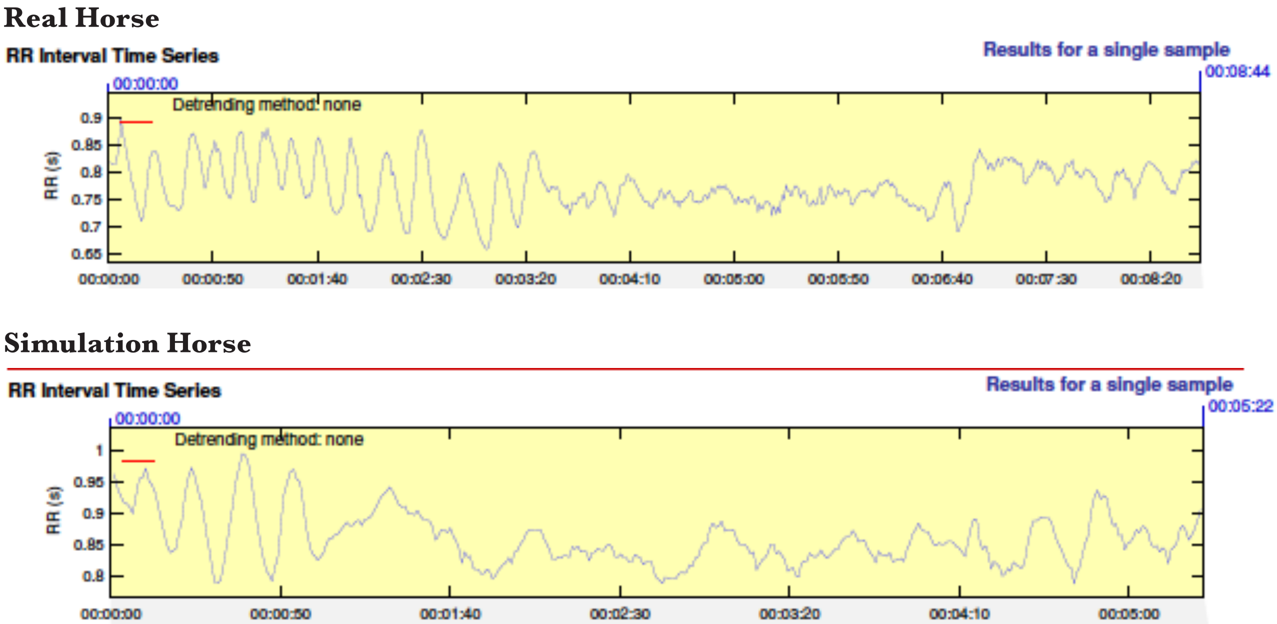

Figure 2. Examples of heart rate variability of human participant during coached alpha breathing followed by *mindful grooming*.

during the interactions were transitory. However, after grooming the simulation horse, human participant HR decreased compared to the baseline value, on average (Table 3[b]).

When HRV data were compared between participants who interacted with the horse first vs. the simulation horse first, there was no significant difference. Therefore, it is unlikely that the HRV experience with the horse on day 1 influenced the HRV experience with the simulation horse on day 2 and vice versa. During interactions with horses, but not with the simulation horse, the human HRV frequency spectrum shifted significantly from the LF and HF ranges to the VLF range (%VLF pre vs. during, normal distribution, paired t-test, $t = -4.274$, $p < 0.001$) (Table 3[a]) where the horses showed their strongest contribution to HRV. When grooming the simulation horse there was a non-significant increase in %VLF (%VLF pre vs. during, normal distribution, paired t-test, $t = -1.345$, $p = 0.195$) (Table 3[b]). There were also significant decreases in %HF compared to baseline when grooming the real horse and the simulation horse (real horse: normality failed, Wilcoxon signed-rank test, Z-statistic = −3.245, p = 0.001; simulation horse: normality failed, Wilcoxon signed-rank test, Z-statistic = −3.058, p = 0.001).

For the 20 participants who groomed the simulation horse in the university office remote from any horses, there was no significant increase in HR during the interaction compared to baseline (Table 4). Likewise, there was no significant reduction in %HF. Unlike the results for participants who groomed the simulation horse at the EquuSatori Center, %VLF did not trend toward an increase during the interaction. No postinteraction data are presented in Table 4 because there was an equipment malfunction for two of the participants during those measures, resulting in missing data.

During mindful grooming, do older people become aware of bodily sensations that are overall pleasant?

Exit Interview Words Comparison. Word usage describing interactions with the real horse and the simulation horse reflected significantly more positive than negative sensations in both cases (horse interaction: normal distribution, paired t-test, $t = 5.175$,

Table 3 Heart Rate Variability of Humans Participating in EAL Exercise, Mindful Grooming, with a Real Horse (a) or a Simulation Horse (b) (the same people participated in both sessions)

a.	Pre	During	Post
HR (bpm)	75.0 ± 10.1 (SD)	81.2 ±11.9*	75.2 ± 12.0
SDRR (ms)	36.5 ± 17.8	58.6 ± 26.1*	33.9 ± 12.5
RMSSD (ms)	26.3 ± 37.1	19.7 ± 17.3	18.9 ± 18.3
% LF	33.9 ± 17.8	24.7 ± 16.8*	34.7 ± 15.5
% HF	16.1 ± 19.7	4.8 ± 4.5*	12.7 ± 12.9
% VLF	50.1 ± 17.8	71.0 ± 19.2*	52.7 ± 17.6

p-values	Pre vs. During	Pre vs. Post
HR (bpm)	0.001	0.729
SDRR (ms)	0.001	0.348
RMSSD (ms)	0.829	0.490
%LF	0.034	0.793
%HF	0.001	0.154
%VLF	0.001	0.373

b.	Pre	During	Post
HR (bpm)	77.6 ± 10.3 (SD)	78.4 ±10.6	74.6 ± 10.2**
SDRR (ms)	34.4 ± 17.7	46.2 ± 14.7*	39.2 ± 17.7
RMSSD (ms)	24.0 ± 36.2	24.2 ± 23.8	29.7 ± 30.1
% LF	31.1 ± 19.3	31.5 ± 14.5	32.3 ± 17.2
% HF	15.5 ± 19.2	5.9 ± 6.6*	19.3 ± 21.5
% VLF	53.5 ± 21.3	62.7 ± 16.8	48.3 ± 25.1

p-values	Pre vs. During	Pre vs. Post
HR (bpm)	0.181	0.001
SDRR (ms)	0.008	0.087
RMSSD (ms)	0.481	0.169
%LF	0.479	0.733
%HF	0.001	0.768
%VLF	0.195	0.358

Note: Single asterisks represent significant difference between values pre and during intervention. Double asterisks represent significant difference between values pre and post intervention.

Table 4 Heart Rate Variability of Humans Participating in EAL Exercise, Mindful Grooming, with a Simulation Horse in an Office

	Pre	During	*p*-value
HR (bpm)	75.5 ± 10.4 (SD)	77.6 ± 9.0	0.073
SDRR (ms)	31.2 ± 14.2	46.8 ± 27.3*	0.035
RMSSD (ms)	22.0 ± 22.0	39.7 ± 46.8	0.391
% LF	35.4 ± 16.9	31.1 ± 13.8	0.423
% HF	15.5 ± 14.2	20.3 ± 21.2	0.433
% VLF	49.1 ± 22.4	48.5 ± 27.6	0.941

Table 5 Valence of Word Usage Describing Positive, Neutral and Negative Feelings and Sensations Evoked by Interactions with the Toy Horse and the Real Horse

	Positive	Neutral	Negative
Simulation	4.75 ± 4.17* (SD)	1.83 ± 1.88	1.25 ± 1.19
Horse	4.83 ± 3.32*	2.63 ± 2.06	0.87 ± 1.19

*Number of positive words significantly greater than number of negative words for both simulation and real horse.

$p < 0.001$; simulation horse interaction: normal distribution, paired *t*-test, $t = 3.975$, $p < 0.001$, Table 5). Grooming the real horse evoked slightly more positive and neutral words than grooming the simulation horse, and slightly fewer negative words. However, these differences were not statistically significant.

Exit Interview Gestures Comparison. For both the simulation horse and real horse, positive gestures were used the most during the exit interview, while negative gestures were used the least (horse interaction: normal distribution, paired *t*-test, $t = 6.808$, $p < 0.001$; simulation horse interaction: normal distribution, paired *t*-test, $t = 4.169$, $p < 0.001$, Table 6). These results were similar to those for word usage. However, analysis of the gestures did reveal a difference between those made while talking about grooming the real horse compared to the simulation horse. On average, grooming the real horse evoked

Table 6 Valence of Gestures Expressing Positive, Neutral and Negative Feelings and Sensations Evoked by Interactions with the Simulation Horse and the Real Horse

	Positive	Neutral	Negative
Simulation	11.21 ± 4.62*+ (SD)	10.46 ± 6.37	5.80 ± 3.50
Horse	14.29 ± 7.00*	11.67 ± 5.58	3.71 ± 3.18

*Number of positive gestures significantly greater than number of negative gestures for both simulation and real horse
+Number of positive gestures for real horse is significantly greater than for simulation

significantly more positive gestures than grooming the simulation (normal distribution, paired *t*-test, $t = -2.300$, $p = 0.031$). In addition, significantly more negative gestures were used when the participants discussed grooming the simulation horse versus the real horse (normality failed, Wilcoxon signed-rank test, Z-statistic = −2.12, $p = 0.036$).

Does HRV in horses remain within the normal range during unmounted EAL?

Heart rate for all three horses remained in the normal range for horses at rest (28–48 bpm, from Rutgers New Jersey Agricultural Experiment Station Cooperative Extension Fact Sheet 1262), throughout the experiment (Table 7). Regarding HRV, measures of SDRR made prior to mindful grooming were low for Opalo and Sunday compared to previous measures on horses trained for EAL (Baldwin et al., 2018; Gehrke et al., 2011), but in both cases SDRR increased during grooming. Prescott already showed normal SDRR at baseline and this value remained the same during grooming. All three horses maintained high levels of %VLF (over 70%) throughout the experiment, which is usual for EAL horses at rest (Baldwin et al., 2028; Gehrke et al., 2011). Further analysis of the HRV frequency domain revealed that before and/or during interactions with humans all three horses showed a combination of several of the following HRV oscillation peaks (Hz): 0.002, 0.003, 0.004, 0.005, 0.007, 0.008, 0.015, which are all within the VLF range. These results indicate that

Table 7 Heart Rate Variability of Three Horses Participating in EAL Exercise, Mindful Grooming

Opalo	Pre	During	Post
HR (bpm)	40.1 ± 2.5 (SD)	41.3 ± 2.9	40.8 ± 2.4
SDRR (ms)	61.5 ± 5.5	82.8 ± 14.7	51.6 ± 13.2
%VLF	87.8 ± 3.4	88.3 ± 4.1	87.7 ± 3.4
Sunday	**Pre**	**During**	**Post**
HR (bpm)	38.0 ± 2.8 (SD)	33.7 ± 1.0	32.2 ± 1.2
SDRR (ms)	83.0 ± 11.0	125.1 ± 16.4	93.9 ± 10.4
%VLF	76.3 ± 20.3	83.3 ± 6.2	86.1 ± 11.8
Prescott	**Pre**	**During**	**Post**
HR (bpm)	36.7 ± 2.4 (SD)	44.6 ± 12.0	50.2 ± 16.1
SDRR (ms)	164.7 ± 22.5	162.3 ± 34.4	186.2 ± 11.6
%VLF	90.3 ± –11.7	86.1 ±11.8	91.5 ± 4.1

Note: Data are based on measurements from 8 sessions with each horse.

the horses were not stressed by the procedure and were suitable for mindful grooming.

Does participation of horse-human pairs in mindful grooming cause synchronistic changes in HRV?

In 10 out of 24 human/horse pairs, matching HRV oscillation frequencies occurred during the interaction that were not present during pre-interaction measures (Table 8). These data support the concept that oscillations of HRV may became synchronous between horse and human during *mindful grooming*.

Table 8 Human/Horse Pairs Showing Matching HRV Frequencies During Mindful Grooming

Human/Horse Names	Matching Frequency (Hz)
Barbara/Opalo	0.002
Jon/Opalo	0.012
Debrah/Sunday	0.002
Sabrina/Sunday	0.002, 0.007, 0.011
Twila/Opalo	0.015
Adrienne/Sunday	0.008
Beth/Sunday	0.006
Bill/Prescott	0.001
Erica/Prescott	0.005

Discussion

During mindful grooming, do older people experience an increase in HRV?

The results show that when older people participate in mindful grooming their HR and HRV significantly increase compared to baseline values, similar to our previous findings (Baldwin et al., 2018). These studies, in addition to others (Gehrke et al., 2018; Ecker & Lykins, 2019), indicate that humans generally respond positively when engaging with horses in EAL settings.

While each participant was coached in alpha breathing, a wavelike pattern occurred in the HRV recording. This response was due to the strong respiratory contribution to HRV. When people breathe slowly, at about 5–6 breaths per minute, there is a noticeable increase in HR during the inhale and a decrease during the exhale. This is a normal, healthy response due to changes in ANS sympathetic/parasympathetic balance and greatly enhances HRV (Yasuma & Hayano, 2004). Interestingly, although the participants were instructed to continue the alpha breathing while grooming the horse, the wavelike HRV rhythm disappeared when the participant started grooming the horse. It is likely that when participants were grooming, their attention to deep, slow breathing faded, thus reducing the respiratory contribution to HRV that is normally responsible for the wavelike signal.

During mindful grooming, do older people become aware of bodily sensations that are overall pleasant?

When engaging in mindful grooming, people are encouraged to direct their awareness to their bodily sensations so they become conscious of how a healthy, mildly aroused physiological state feels within their own body. Analysis of the exit interviews provided rich evidence that engaging in mindful grooming of both the real horse and the simulation awakened the participants' awareness of bodily sensations that were mainly pleasurable. One variable that may have influenced these results is the environment. Equine-related smells and sounds that surrounded the participants during each interaction may have influenced the participant even during the grooming of the simulation horse. However, grooming the real horse added something extra as evidenced by a significant increase in the number of positive gestures made by participants during their exit interviews. This result implies that the bodily sensations experienced while grooming the real horse were significantly more pleasant than those felt while grooming the simulation.

Does HRV in horses remain within the normal range during EAL?

As the popularity of EAL grows, it is important to understand not only the contribution horses make to human well-being, but also to ensure that they remain healthy. This study and others (Baldwin et al., 2018; Janczarek et al., 2018; Malinowski et al., 2018; Mendoca et al., 2019) showed through HRV measurements, that the horses are not stressed but are neutral to, or positively affected by the interactions. In conclusion, most studies published so far indicate that interacting with a live horse, with the guidance of an EAL professional, does not adversely affect the horse and usually increases human HRV, reflecting healthy heart-brain neural communication and sympathetic/parasympathetic autonomic balance.

This study and others (Baldwin et al., 2018; Gehrke et al., 2011; Institute of HeartMath, 2006) show that when horses are in a relaxed state, they naturally generate a high %VLF in their HRV frequency spectrum. In the present study all three horses showed a combination of some of the following HRV oscillation peaks (Hz): 0.002, 0.003, 0.004, 0.005, 0.007, 0.008, 0.015. These frequency peaks are all in the VLF zone that is associated with relaxation in horses. In our previous study (Baldwin et al., 2018), performed on three horses in Arizona, the peak frequencies observed were very similar to those of the horses in the present study: 0.002, 0.003, 0.004, 0.005, 0.006, 0.012, 0.032 Hz. Further studies performed on different horses in various locations have yielded similar results. It appears that a high %VLF is an autonomic characteristic of horses when relaxed and at ease.

Does participation of horse-human pairs in mindful grooming cause synchronistic changes in HRV?

Previous studies (Baldwin et al., 2018; Gehrke et al., 2011) show that horses in the resting state have a higher %VLF power in their HRV spectrum than is usual for humans. In this study the human HRV frequency spectrum significantly shifted to the VLF range, similar to horses, as participants engaged in mindful grooming with the live horse, but not as they groomed the simulation horse. Although an increase in %VLF has also been associated with physical activity in humans (Bernadi et al., 1996), this does not explain our findings. When our participants groomed the simulation horse, they experienced the same mild physical activity as grooming the real horse, but no increase in %VLF was observed. Why is a shift to the VLF frequency range of HRV beneficial to humans? Experimental evidence suggests that people who have only a small %VLF contribution to their overall HRV are more prone to death after cardiac arrhythmia (Bigger et al., 1992), inflammation (Lampert et al., 2008), and post-traumatic stress disorder (Shah et al., 2013). All these issues are important clinical considerations in older adults.

Apart from a general shift of the human participants' HRV frequency spectrum to the VLF range, there was also specific coupling of HRV frequency peaks in that range between horse and human in 10 out of 24 horse-human pairs. It is unlikely that this was just a characteristic of the grooming process because similar coupling was observed in 7 out of 24 horse-human pairs in a previous study using a different EAL activity (Baldwin et al., 2018). We are not the only investigators to have noticed this phenomenon. Lanata et al. (2016), working with 11 human participants and 1 horse, showed that when the human and horse were in the same stall, there was increased coupling between their heartbeat dynamics compared to when they were in two separate stalls. These authors hypothesized that the horse and human hearts acted as two biological complex oscillators that were becoming coupled over time. An associated group of authors has hypothesized that when a horse and a human experience cardiovascular coupling during an interaction, there is also mutual coordination of emotional states (Scopa et al, 2019). Similar synchronization of HRV has been observed between humans. McCraty (2004) observed that entrainment (phase or frequency locking) of the HRV patterns between individuals is more likely to occur if the pair have a close working relationship or live together in a bonded relationship. In addition, McCraty (2017) states: "Various studies examining synchronization between mothers and infants, pairs and groups, indicate that feelings of cooperation, trust, compassion and pro-social behaviors are facilitated by physiological synchronization between individuals." More research is required to test whether horses and humans may connect and communicate through cardiac entrainment.

Strengths of This Study

The main strength of this study is that it is one of only three EAL studies involving simultaneous measurement of HRV in horses and humans. Simultaneous measures are necessary for two reasons. First, the data indicate whether the level of arousal of a particular horse is being consistently raised or lowered by engaging in a specific EAL activity. This information is important because it could be used, in conjunction with behavioral observations, to assess whether the horse is suitable for a particular EAL activity or for interacting with a particular person. Second, simultaneous measurement of HRV in humans and horses is the only way to explore the possibility of interspecies coupling of HRV dynamics. Comparison of the human HRV results obtained when grooming the real horse versus the simulation horse in the barn and in the office clearly shows that the shift to the VLF range in humans, with its added benefits, is mediated by the presence of a live horse rather than any environmental factors. Another strength of this study is the design and implementation of the protocol. All activities occurred in different parts of the same building (the barn), and participant flow was regulated so that they did not see any horses before starting mindful grooming.

Limitations

One weakness of the study was that the participants were self-selected, lived in a high-income neighborhood, and were all Caucasian. The results may differ for people who are more diverse in ethnicity and income. For example, ethnoracial minorities could show different relationships between emotional regulation and physical health outcomes compared to Caucasians, thus affecting the relevance of EAL in improving health. Regarding income, higher income is related to better health conditions and lower health risks, while lower income means more exposure to health risk factors. More research on diverse populations is needed to determine whether EAL is more or less effective at improving emotional regulation in less healthy people who are already compromised.

A second weakness concerns the effectiveness of HRV as a biomarker for stress in equines. Although the horses all remained within the normal range of HRV for horses at rest throughout the experiment, these data by themselves do not prove that HRV is an effective biomarker for stress in equines. To test this hypothesis, it would be necessary to deliberately subject the horses to known stressors and determine if the HRV significantly decreased, and this was not a goal of the experiment.

A third weakness is that the horses were randomly assigned to participants and so they did not have a choice with whom to work. This was due to the necessity of completing the experimental and control protocols with 24 participants over a short time period. In our previous study (Baldwin et al., 2018), the equine and human participants selected each other through "mutual choosing" in which each participant observed four horses and noticed which one was paying them more attention as indicated by the horse looking at or moving toward them or cocking an ear. The factors influencing mutual choosing are not known but may include bodily scents or body language that both find attractive or may involve HRV coupling. Nevertheless, despite the absence of mutual choosing, the participants in this study showed similar changes in HRV during the EAL activity as in the previous study (Baldwin et al., 2018).

Conclusion

In the present study improvements in both HRV and interoception showed that EAL enhanced autonomic and emotional regulation in older adults. Simultaneous physiological measures in horse-human pairs during *mindful grooming* showed that people aged 55 and older benefit by experiencing increased HRV, which is a marker of improved autonomic balance, along with heightened awareness of pleasant bodily sensations, and often a physiological coupling of their heartbeat dynamics with those of their horse, possibly reflecting emotional bonding. These responses are particularly important for older people because autonomic regulation, sensory awareness, and social interaction can all diminish with age. Measurement of HRV in the horses before, during, and after mindful grooming indicated that they were not physiologically challenged by the process, consistent with other studies. This study provides objective evidence that *mindful grooming* benefits older people, both physically and emotionally, thus lending credibility to EAL practitioners working with this population.

Summary for Practitioners

Equine-assisted learning is often used to improve a person's interoception and physical health. In this study participants engaged in a simple EAL activity, *mindful grooming*, while tuning into their body sensations and observing the horse's response. It was hypothesized that the participants, aged 55 and older, would improve their autoregulation by practicing interoception. Physiological information during horse-human interactions is essential to optimize benefits for horses and humans. Heart rate variability is an established biomarker of autoregulation; higher variability reflects improved cardiovascular function, emotional balance, and social engagement, whereas low HRV may indicate stress-related anxiety and compromised immune function. Heart rate variability is increased by breathing more slowly and by pleasurable sensations, as happens in mindful grooming. Measurements of HRV were made in participants engaging with a real horse and a simulation horse at the EquuSatori Center on consecutive days.

It is important that the horses are not stressed by EAL activities. For this reason, HRV was measured in each horse as well as each human, also showing whether HRV synchronized between horse and human. Exit interviews documented sensations felt by participants during the activity. A separate participant group groomed the simulation horse in an office remote from horses in case nearby horses affected the results.

As hypothesized, when participants groomed a real horse, they became aware of pleasant bodily sensations, their HR and HRV increased, and HRV frequency spectrum shifted to the very low frequency (VLF) range. This range is characteristic of horses at rest and has physiological and psychological benefits for humans. There was synchronization of HRV frequencies within the VLF range in nearly half the horse-human pairs. The horses' HRV remained within the normal range. When participants groomed the simulation horse their HR did not change, and after grooming it significantly decreased, suggesting less autonomic stimulation than when grooming the real horse. In addition, at the EquuSatori Center, HRV increased and %VLF trended upward. This trend disappeared when participants groomed the simulation horse in an office, indicating that horses nearby affected participants' %VLF. Grooming the simulation horse induced pleasant sensations, but they were not recalled as warmly and authentically as those from the real horse.

Further research may determine which EAL activities benefit specific human populations. Simultaneous measurements on horses and humans may determine which protocols best maintain equine autonomic balance and encourage physiological coupling and emotional bonding. Additional research with older people could reveal whether EAL enhances their physical abilities and social skills. Future experiments could also address whether HRV coupling between horse and human is linked to changes in other physiological parameters associated with emotional bonding, such as secretion of oxytocin, serotonin, dopamine, and endorphins. Such correlations would provide biochemical evidence, currently lacking, that HRV coupling is associated with enhanced interoception.

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Authors' Note

This research was approved by the Institutional Research Board and by the Institutional Care and Use Committee and meets the criteria for approval under 45 CFR 46.110, 45 CFR 46.111, and/or 21 CFR 50 and 21 CFR 56. None of the authors have any personal, financial, potential, or actual conflicts of interest.

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