Spring 5-16-2014

Assessment of Horses for Therapeutic Riding Purposes: Comparison of Physiological and Behavioral Parameters

Leah Catherine Turner
Western Kentucky University, leahcatherine.turner181@topper.wku.edu

ASSESSMENT OF HORSES FOR THERAPEUTIC RIDING PURPOSES: COMPARISON OF PHYSIOLOGICAL AND BEHAVIORAL PARAMETERS

A Capstone Experience/Thesis Project

Presented in Partial Fulfillment of the Requirements for

the Degree Bachelor of Science with

Honors College Graduate Distinction at Western Kentucky University

By

Leah Catherine Combs Turner

*****

Western Kentucky University
2015

CE/T Committee:

Dr. Petra B. Collyer, Advisor

Dr. Michael L. Collyer

Dr. Benjamin LaPoe

Approved by

____

Advisor
Department of Agriculture

ABSTRACT

Many accidents in the horse sector presumably happen due to a misinterpretation of horse behavior, and it is difficult to objectively determine the suitability of horses for therapeutic riding programs in regard to their temperament and reactivity. The animals in therapeutic riding programs come from tremendously varied backgrounds and are not always donated with honorable intentions. The horses undergo a training period during which they are evaluated for suitability before they begin working with disabled humans, but there is no requirement that additional desensitizing training be administered past this point. Our study aimed to assess, objectively, a horse's suitability for use in a therapeutic riding program to prevent injuries to participants and to promote animal welfare. Behavioral signs of fear, pain, and mental state were assessed through video footage as well as analysis of salivary cortisol concentration and heart rate variability measurements.

Results indicated that heart rate parameters and behavior tests are accurate, practical, and objective methods of determining horse emotionality and suitability for therapeutic horseback riding, while cortisol analysis, due to time and finances, is not.

Keywords: Therapeutic Riding, Cortisol, Heart Rate Variability, Horses, Stress, Behavior

Dedicated to my parents,

to whom I told I was never going to college

ACKNOWLEDGMENTS

This project would not have been possible without the encouragement, insight, and ambition of many. I am extremely grateful to my advisor, Dr. Petra Collyer, for her eternal optimism, time, constant encouragement, and insight throughout this project. Thanks also to Dr. Michael Collyer and Jordan Vorst for their help and insight in statistical analyses, as well as Naomi Rowland of the Biotechnology center for her invaluable assistance with cortisol analysis.

I would like to thank the Western Kentucky University Honors College for their support throughout my undergraduate studies and for their assistance to achieve a more excellent education and richer collegiate experience. The Office of Scholar Development has been a tremendous asset as well in supporting me through grants, scholarships, and proof-reading documents in pursuit of study opportunities abroad as well as at home. A very special thanks to the Office of Research for their generous FUSE grant and the Honors College for the Honors Development Grant, which were utilized for this research; without these awards this project would not have been possible.

Lastly, endless thank you's to friends and family, all of whom rejoiced with me at the opportunities and rewards attained by this project and wept with me during the stressful times. Especially my father—the eternal librarian, my mother—the heart, and Mrs. Denise Anderson, BA, BS—the associate, friend, and creative genius.

VITA

November 26, 1991.. Born – Hazard, Kentucky

2005... Perry County Central High School,
Hazard, Kentucky

2009... Honors College, WKU

2012... Studied Abroad, Moscow, Russia

2012... Benjamin A. Gilman awardee

2012... Jarve endowed Honors College awardee

2013... FUSE Grant awardee

2014... WKU REACH Conference
Special Selectee

FIELDS OF STUDY

Major Field: Agriculture (Animal Science concentration)

Minor Field: Chemistry

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Therapeutic riding involving equids is not a recent practice, although the exact beginning is uncertain. Evidence suggests that prior to the Common Era, ancient Greeks recognized and incorporated horseback riding to assist people with disabilities. In England, doctors began using horses to rehabilitate soldiers that were injured in World War I, and by the 1950s British physiotherapists were beginning to explore the merits of horse riding as a method of physical therapy.[15] Even greater attention was brought to bear when the Danish equestrian, Lis Hartel, won the silver medal for Dressage in the 1952 Olympic Games in Helsinki. A victim of polio at the young age of 23, horse-enthusiast Hartel was determined that the resulting paralysis of the illness would not deter her from riding. Because of her great success emotionally and physically due to working with her horse Jubilee, Hartel promoted therapeutic riding worldwide, helping to bring attention to this therapeutic tool.[28] Beginning in the 1960s, numerous therapeutic riding programs were formed throughout Europe, Canada, and the U.S., as shown below:

Date	Organization	Mission
1969	Professional Association of Therapeutic Horsemanship International (PATH Int'l) *[originally North American Riding for the Handicapped (NARHA)]* http://www.pathintl.org	Promote safety and optimal outcomes of equine-assisted activities and therapies (EAAT)
1969	Riding for the Disabled Association (RDA) http://www.rda.uk.org	Delivering opportunities for therapy, opportunity, and enjoyment to people with disabilities across the spectrum of disabilities, age range, social status, and urban and rural environments
1970	The Association of Chartered Physiotherapists in Therapeutic Riding (ACPTR) http://acptr.csp.org.uk/about-us	Utilize and develop the professional skills of its members in the areas of therapeutic riding and hippotherapy treatment and management
1980	Federation of Horses in Education and Therapy International AISBL (HETI) *[originally Federation of Riding for the Disabled International (FRDI)]* http://www.hetifederation.org	Facilitate worldwide collaboration between organizations and individuals whose objectives are philanthropic, scientific, and educational in the field of EAAT
1992	American Hippotherapy Association (AHA) http://www.americanhippotherapyassociation.org	Provides education, facilitates research, and promotes equine assisted therapy as an effective treatment strategy that improves the quality of life with individuals with disability
1997	Eponaquest http://eponaquest.com	Horse training, breeding concepts, and programs to educate about horses and leadership, personal, relationship skills
1999	Equine Assisted Growth and Learning Association (EAGALA) http://www.eagala.org	Bring public awareness to health treatments of equine assisted therapy, give access to funding programs, training professionals to work in the EAAT field
2004	European Association for Horse Assisted Education International (EAHAE) http://www.eahae.org	Establish and develop horse assisted education for enterprises, institutions, and personal purposes
2004	Horses and Human Research Foundation (HHRF) http://www.horsesandhumans.org	To support, promote, and fund scientific research for claimed, yet unsubstantiated benefits of EAAT; to educate the public on research
2007	Equine Experiential Education Association (E3A) http://www.e3assoc.org	Training, certification, and resources for implementing EAAT

While each organization offers a unique perspective on equine-assisted activities and therapies (EAAT), what exactly are the merits of such a therapeutic tool? Therapeutic riding assists individuals of all age groups with many different disabilities; for those with physical disabilities, the warmth and movement of the horse's body can reduce spasticity and enhance coordination in muscle groups within the legs and other parts of the body. Head and body control can improve as a result of the rider needing to pay attention to where the horse is moving, and manipulating the horse's reins grants upper extremity and hand control. Besides these benefits, the horse's movement provides the rider's neuromuscular system with movements that are similar to those provided during normal human ambulation (walking).[8] Individuals with a cognitive disability can observe and participate in the stimuli allowed by the riding lesson and learn to focus and follow directions. Lastly, an individual with any emotional disability can benefit from the formation of a special relationship with the assigned horse, which ultimately can lead to greater self-esteem, patience, and trust.[10] Specifically, multiple studies have been conducted concerning the effects of EAAT for children with cerebral palsy,[6,13,7] which consistently demonstrate EAAT efficacy; research concerning the merits of EAAT for individuals with various cognitive or mental disabilities also support effectiveness of this therapeutic tool.[20,32,11,9] A typical therapeutic riding session will include sensory integration, for those who are physically able, to brush and tack the horse. Riding sessions are directed by the therapist according to each rider's individual needs, and will usually conclude after 30-60 min; therapists will address rider impairments, functional limitations, and disabilities by having riders change position and instigate a change in horse speed and direction. These changes will stimulate different muscles in the rider due to weight shift response in order to stay balanced atop the horse, therefore increasing muscle tone and response. Other non-physical benefits of riders include improvement in volume and quality of vocalization (such as telling a rider to direct the horse with “walk,” “stop,” “back” commands), encouraging the rider to explore and touch (such as asking the rider to reach up to pet the horse’s neck), and lengthening attention span (such as having the rider participate in games like finding objects in the arena and bringing them back to the instructor).[34]

EAAT and therapeutic riding are therapeutic tools ever-growing in usefulness and popularity; as a result, it is important that these programs be designed to be as safe and efficient as possible. This study aimed to improve the experience and safety of therapeutic riders, but also to improve equine welfare by laying foundations for objectively determining horses best suited for this niche of work.

CHAPTER 2

LITERATURE REVIEW

1. Behavior Tests and Ethograms

Because therapeutic riding programs are dealing with a physically disabled general public, it is important that the programs be able to implement safety in their lessons. While PATH recommends a trial period for any potential therapy horse, they do not specify how preliminary selections with regard to temperament and reactivity can be made—only suggestions on what characteristics to look for.[12] The National Electronic Injury Surveillance System of the U.S. Consumer Product Safety Commission reports that 64,693 equine accidents occurred in the year 1998,[33] and a Cambridge University study surveying 1,000 riding accident hospital admissions showed that one injury occurs for every 100 hours of leisure riding, one injury for five hours of amateur jumping events, and one injury for every hour of cross-country eventing.[31] In an Australian Study, horse behavior was the highest single cause of injury.[33] Because a fair amount of accidents occur in the equine sector, presumably due to horse behavior, an objective and consistent test for emotionality and behavior of potential therapy horses must be conceptualized. Several studies have been conducted on this matter[3,22,23,36] using behavioral tests and/or questionnaires given to caretakers or novice riders to complete; in their study, "Responses of horses in behavioural tests correlate with temperament by riders",Visser et al.[36] concluded that the temperament of a horse, as determined by behavioral tests, correlated significantly with temperament assessed by a group of novice riders who had never been in contact with the horses before. Despite this, they believe behavioral tests have a number of advantages, such as there is no need for a large gathering of assessors, each horse is being subjected to the same treatment in the same setting (more control), and data gathered are more quantitative so that data are more comparable within the study and between studies. Behavioral tests include reactions to a novel object (balloons, toy, open umbrella, etc.), unknown handler, social isolation, food isolation, and open-arena; these tests are often correlated with physiological responses, such as heart rate variability and cortisol levels in blood, urine, feces, or saliva.

Behavioral tests are interpreted with an ethogram, an inventory of behaviors explained and catalogued in order to reduce subjectivity of the tests. While PATH states that a trial period must be conducted for every horse inducted into a therapeutic riding program, there is no mention of annual retraining or conducting more than one trial period.[12] In the publication, "Behavioral assessment of horses in therapeutic riding programs," Anderson et al.[3] strongly suggest that yearly desensitizing training be administered to therapeutic riding horses in order to ensure a safe environment for disabled and novice riders. In their study, three novel objects were used to determine emotionality and reactivity of horses: Twenty-two horses came from the Texas A&M University (TAMU) Horse Center, three from the TAMU therapeutic riding program, eight were privately owned and had never participated in therapeutic riding, and seventy-three horses were selected from five separate therapeutic riding centers. It was found that 64% of the tested horses that had the highest reactivity scores were therapeutic riding horses—and the horse with the highest reactivity score was a therapeutic riding mount that had been used in a therapeutic riding program for two years.

Because of varying literature results concerning the validity of questionnaires versus behavioral tests, utilizing both modalities is considered the best practice when determining emotionality of horses to be used in therapeutic riding programs, and a yearly desensitizing training administered to reduce the number of accidents regarding equine assisted therapies.[22]

2. Cortisol and Heart Rate Variability

Two methods for determining stress levels of a horse that have become the most widely accepted are testing cortisol levels from body fluids (in blood, urine, feces, or saliva) and heart rate variability (HRV); cortisol is a steroid hormone that is released from the adrenal cortex in response to stress. Collecting salivary cortisol determines the unbound fraction of this hormone in the saliva. This has proven to be as accurate as more invasive procedures, such as cortisol extracted from blood plasma.[17,26] To obtain and measure salivary cortisol, a swab is taken from inside the mouth, and ultimately measured using assays to determine concentration of cortisol in the salivary sample.[25]

HRV is used to measure the variation in time between heart beats. Essentially, HRV is a measure of the balance between the parasympathetic nervous system (part of the autonomous nervous system that regulates bodily functions while the animal is at rest) and the stress related (fight or flight) related sympathetic nervous system.[27] Recognition that living beings possess a pulse that beats at various intervals, and is controlled by the heart, has existed for centuries, yet the use of HRV in scientific study was not practical until the early 20th century. The electrocardiogram (ECG) is a device that is made up of a positive and negative electrode. These are attached to the outside of the human/animal body in order to measure electrical impulses generated by cardiac tissue resulting in a multi-peak wave. HRV has been a major contributor to numerous studies conducted regarding correlations between stressful situations, poor health, mental disorders and HRV in both animals and humans.[2,14,19,21] Utilizing HRV is a commonly accepted method to determine stress levels and temperament of horses.[29,38]

While behavioral tests and questionnaires are adequate tools to assess a horse's personality, or reactivity, HRV and measuring cortisol levels provide a more quantitative and objective approach to research concerning horse emotionality or temperament. Multiple studies have been conducted confirming the success of these two methods, and a high correlation between data retrieved from these measurements and more subjective methods, such as questionnaires and behavior tests, lend credence to their efficacy.[30,38,4,1

CHAPTER 3

MATERIALS AND METHODS

This study was approved by Western Kentucky University's Institutional Animal Care and Use Committee (Animal Welfare Assurance #A3558-01, designation 13—13).

1. Animal Housing and Management

A total of 12 horses (4 mares and 8 geldings) of various breeds (2 Appaloosas, 8 Quarter Horses, 1 Quarter Horse cross, and 1 Thoroughbred) were used for this study. The horses were 5–21 years old, and had been involved in the therapeutic riding program for the duration of 1-16 years. The horses came from diverse backgrounds, and were previously used either as trail, dressage, barrel, racing, jumping, or as pleasure horses in the greater Bowling Green area. The horses were turned out to pasture for the predominance of each day (approximately 23 hours per day) except for when brought in for riding lessons or during extremely cold weather. When off pasture, horses were housed individually in 3.66 m x 3.66 m straw-bedded stalls, and had access to water; they were fed approximately 0.907–1.81 kg of grain twice daily, regardless of whether they were used for a riding lesson or not; horses were only used for therapeutic riding and individual riding lessons, which averaged approximately 1.5–2 hours of work per day, and were exercised on average for 2 hours per week.

2. Behavioral Tests

2.1 Separation Test

This test was conducted in order to test each horse's behavior when placed in an isolated environment. In non-social species, the novel environment test is used to observe anxiety and fear. In horses, as a social species, this behavioral test is used to observe separation anxiety and gregariousness. Each horse was directed into a 30.5 m^2 arena, equipped with heart rate monitor electrodes attached to a training surcingle, and videotaped for exactly five minutes.

2.2. Novel Object Test

This test was conducted in order to ascertain each horse's behavior when confronted by an unfamiliar object; the degree of fearfulness was tested. Each horse was re-directed into the same 30.5 m^2 arena as during the separation test, with the heart rate transmitter still strapped to the girth, and videotaped for exactly five minutes. Approximately ten seconds after the doors of the arena were closed and the horse was isolated within, a 1.83 m colorful umbrella was raised into the air for the remainder of the five minutes.

2.3. Novel Object/Novel Handling Test

This test was similar to the novel object test; the difference here was that a handler was involved, who directed the horse as passively as possible to the novel object. This test, in our study referred to as the "bridge" test, was conducted in order to test a horse's behavior when directed to walk, and stand for a brief period, onto an unfamiliar object. For this test, we used a 1.83 m x 0.914 m wooden bridge covered in pink bubble wrap as our novel object. The heart rate transmitter was still strapped to the horse's girth in order to ascertain heart rate activity and video footage filmed for later analysis. Each horse's handler was instructed to walk towards the bridge, passively holding the horse's lead rope, then walk next to the bridge to assess whether the horse would willingly walk onto the bridge or not. Several attempts were allowed; the only stipulations placed onto the leaders were that if the horse balked at the bridge to turn them around and make another attempt, not to exert any pressure on the lead rope to persuade the horse to walk atop the bridge, and when/if the horse did walk onto the bridge to stop and have the horse stand still for three seconds while still on the bridge.

2.4 Riding Lessons

The riding lessons were observed in order to be able to compare the information we obtained from the behavioral tests, which were each designed specifically to stress the horses out in a certain way, in order to ascertain whether the horses would show stress during their "every day" work like they would during the behavioral tests. Each lesson conducted followed this pattern: the horse was lead into the holding stall and placed into cross-ties; each child was expected to assist in the grooming of the horse, to the best of his or her ability; also, if able, the child is expected to assist in placing the saddle blanket and saddle on the horse; once the horse is groomed, saddled, and bridled, the child will mount the horse and proceed with the riding lesson, which was tailored specifically for each child's individual needs, for approximately thirty minutes; the child would then dismount and again assist as much as he/ she were able in removing the saddle and saddle blanket and post-ride grooming. For this part of our study, two cameras were placed on opposite sides of the arena on tripods, and the section of the riding lesson wherein the child was mounted and actually riding, was recorded. The heart rate transmitter was affixed to each horse immediately upon being placed in the cross-ties of the holding stall, and removed upon returning to the holding stall and being unsaddled.

3. Ethograms Constructed for Evaluation

Label	**Definition**
Canter	Cantering
Trot	Trotting
Rel_walk	Head is at/below withers; fairly slow gait; absence of extreme alertness or distress
Vig_walk	Head above withers; tense/fast-paced gait; ears pricked; nostrils flared
Stand	Standing without tenseness; head at/below withers
Stand_alert	Standing with ears pricked; nostrils flared; some sign of alertness
Stand_gate	Standing near/at gate; focused on gate
Stand_people	Standing near people; focused on people; relaxed
Graze	Grazing/attempting to graze
Pawing	Pawing
Whinny	Whinnying
Snort_rel	Snorting while in relaxed posture
Snort_alert	Snorting while in alert posture
Chewing	Lips appear to be moving in a chewing motion; licking of lips; not while grazing
Startled	Is startled, jump
Bucking	Raising the back half of the body into the air in an agitated manner
Rearing	Raising the front half of the body into the air in an agitated manner
Push_gate	Pushing at the gate with head/nose/hooves/body
Defecation	Defecates
Urination	Urinates
Rolling	Kneels on ground and proceeds to roll over

Label	Definition
Flehmen	Sniffing an area then curling the upper part of the muzzle over the nostrils
Sharp_turn	While moving in one direction abruptly making a 180° turn
time inner circle	horse within 3 m of novel object
touch NO	number of times horse touching novel object

3.1 Ethogram for Arena-and-Novel Object Tests

Label	Definition
On_bridge_y/n	Did/did not step onto the bridge
Attempts_bridge	Number of times until did step onto bridge
Stop at approach	Stopped before walking onto bridge
Stand_3sec_on_bridge_y/n	Did/did not stand on the bridge for 3 seconds

3.2 Ethogram for Bridge Test/ Novel Handling Test

Label	Definition
Tail Swish	Repeated lateral movements of the tail
Ears Back	Pinning ears to head
Defecation	Defecates
Head Toss	Toss the head into the air once before returning to relaxed position
Head Shake	Tossing the head into the air repeatedly in short period of time
Head Turn	Evading the bit by turning head away from pressure
Lowering/Extending Head	Lowering or extending the head below the withers

3.3 Ethogram for Riding Lessons

4. Physiological parameters

4.1. Cortisol sampling procedures

Salivary cortisol samples were obtained at rest before and after the three behavioral tests, as well as before and after therapeutic riding lessons for each horse. Before completing his/her meal, each horse's mouth was swabbed with a Salimetrics® children's swab for approximately 30 seconds underneath the tongue at least 10 minutes before the behavioral tests/riding lessons, and then swabbed again immediately following the tests/lessons. The swabs were placed in Salimetrics® swab storage tubes and placed on ice in an insulated cooler. Within four hours, the samples were placed into a freezer (-4°C) until all the samples for the study had been obtained and were thawed for analysis.

The samples were analyzed using the ELISA cortisol salivary immunoassay kit by Salimetrics®, a competitive immunoassay specifically designed and validated for the quantitative measurement of salivary cortisol. The test principle was followed according to the kit instructions. A microtitre plate was coated with monoclonal antibodies to cortisol; cortisol in standards or unknowns would compete with cortisol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components were washed away. Cortisol peroxidase that had bonded was measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction would produce a blue color. A yellow color would form after the reaction was stopped with sulfuric acid. Optical density was read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected, which was measured by the intensity of the color, was inversely proportional to the amount of cortisol present. On the day of analysis the samples were thawed completely, vortexed, and centrifuged (Forma 5682) at 3000 rpm for 15 minutes, which removed mucins and other particulate matter that could interfere with antibody binding. While the samples were given approximately an hour and a half to come to room temperature, a 1X wash buffer was made by diluting wash buffer concentrate 10-fold with room-temperature deionized water (100 mL of 10X wash buffer to 900 ml of deionized water). The plate layout on the 96-well-plate was determined. 25 µL of standards, controls, and unknowns were pipetted into the appropriate wells (triplicates); 25 µL of assay diluent were pipetted into two wells that served as a zero value; 25 µL of assay diluent were pipetted into each NSB well. The conjugate was diluted by combining 15 µL of the conjugate with 24 mL of the assay diluent. Exactly 200 µL of this solution was added to each well with a multichannel pipette. The plate was then mixed on a rotator for 5 minutes at 500 rpm and incubated at room temperature for 55 minutes. The plate was washed 4 times with the 1X wash buffer. To each well, 200 µL of TMB were added via multichannel pipette, and the plate mixed on the rotator once more for 5 minutes at 500 rpm and incubated at room temperature, in the dark, for 25 minutes. Fifty µL of stop solution were added with a multichannel pipette, and then the plate was mixed on a rotator for 3 minutes at 500 rpm. Afterwards, the bottom of the plate was wiped with a lint-free cloth until dry, and then read in a plate reader (Biotek Synergy H1 Hybrid Reader with Biotek Gen 5 Data Analysis software) at 450 nm.

4.2. HRV testing procedure

In order to determine heart rate parameters before and during each riding lesson and behavioral test, the Polar Equine RS800CX Science Heart Rate Monitor (Polar, Warminster, PA, USA) was utilized; there were two parts included with the monitor—the electrode transmitter and the wristwatch receiver. The electrode transmitter consisted of two electrodes, which perceived the heart rates, connected by a wire belt that also contained a heart rate sensor, which sent the heart rate data to the wristwatch receiver. The electrodes were placed slightly below the withers and behind the elbow of the horse on the chest, respectively. We affixed the transmitter to the horse via a training surcingle, while thoroughly soaking the area of the horse’s body where the electrodes were going to rest upon, as well as lubricating the electrodes individually with KY jelly; this ensured adequate contact of the electrodes with the skin for more accurate heart rate measurement. The transmitter was affixed to the horse, as described above, while the horse was standing in cross-ties in a holding stall in order to determine a baseline reading for each individual. The wristwatch receiver was formatted such that baseline, individual behavioral tests, and riding lessons heart rates for each horse were not combined so that later analysis could be constructed successfully for each individual (using Polar ProTrainer 5 Equine Edition software). After baseline values were recorded for each horse, individually the horses underwent each behavioral test with the electrode transmitter affixed to them throughout; the same procedure was conducted for the riding lessons. All the heart rate data that was received by the electrode transmitter during each test and lesson and sent to the wristwatch receiver were later downloaded onto a computer and used for analyses such as SDRR, rMSSD, and mean heart rates.

5. Statistical Analysis

Statistical methods utilized for comparison of data were paired t-tests and Pearson correlation; the objective of these tests is to determine the significant changes between two variables, expressed as the probability of determining such changes if the null hypothesis is true. A lower probability would mean that it is rare to observe such change, expressed as t-values, by chance, and thus the results (changes) would be significant. A significant difference between two variables would manifest as a value smaller, or equal to, 0.05. Bonferroni correction was applied to these analyses in order to correct for error of multiplicity—that is, the more variables and number of hypotheses are present in data, the likelihood of witnessing rare event increases, as does the chance to reject the null hypothesis when it is actually true.

A paired t-test is typically used when there is one measurement variable and two nominal variables that are correlated—such as a "before" and "after" of the measurement variable (HR). This test assumes that the differences between pairs are normally distributed. A value, D, is calculated for each measurement value undergoing "before" and "after" treatment (HR at baseline and riding lesson/ behavior test)—then the average, M_D, of these values are calculated. The variance, $SS_{D,}$ of the variables are calculated by summing individual HR values minus the M_D squared. The total number of subjects tested is N. Thus, the equation for calculating t is: $t = \frac{MD}{\sqrt{\frac{SSD}{N}}}$

The t value obtained allows us to look up a p-value associated with the obtained t value using degrees of freedom (df). Df are the total number of subjects tested minus one. The p-value will address how likely it was, considering your null hypothesis being true, for your t-value to be as large as it is. A cut-off point must be chosen for the p-value; this is typically 0.05 or 0.01. For our purposes both were assessed. Ultimately, if between each variable (HR at baseline/ riding lesson/ behavior tests) there was a t-value that corresponded with a p-value of great than 0.05 or 0.01, there was no significant difference between said variables.

Correlated Variables

Pearson's Correlation test was conducted for each heart rate parameter and cortisol in order to determine if there was a relationship between the constructed ethograms and the aforementioned physiological parameters of stress. This test is a measure of the linear relationship, or correlation, between two variables. A calculated Pearson's r-value is how we determine correlation. A value of -1 would mean a directly negative correlation, wherein when one variable decreases the other increases; a value of 0 would mean there is no correlation between the two variables; a value of +1 would mean there is a direct positive correlation between the two values, wherein when one variable increases the other variable does as well. Pearson's r-value is calculated as such: $r = \frac{\sum xy}{\sqrt{\sum x^2 \sum y^2}}$ where $\sum$ is summation, and x and y are the two variables being assessed. The more correlated variables are, the more likely it is that results achieved are significant. Each filter type for HRV (no filter, very low filter, medium filter, very high filter) was tested since no significant differences were found between filter types. Pre- and post- values of SDRR and rMSSD were compared using the equation *Natural Log Difference*=ln*(post)*- ln*(pre)*, which normalized the data and approximated the percent difference. Lastly, each variable had to be equalized to the number of observations of heart rate parameters; in order to do this, the percent of evasive actions were duplicated four times. SDRR, rMSSD (using each filter), and BPM variables were found to have significant correlation with ethogram results (Table 10), while cortisol was not.

CHAPTER 4

RESULTS

Cortisol

Salivary cortisol samples were obtained from each horse before and after the three behavior tests as well as before and after the riding lessons (Table 1). For most horses, cortisol levels rose between the beginning and end of both RL and behavior tests (Fig. 1).

	Pre-RL	**Post-RL**	**Pre-tests**	**Post-tests**
Amber	0.08981	0.06541	0.05183	0.11663
Bennett	0.01439	0.01602	0.05043	0.07703
Chelsea	0.29340	0.05507	0.04467	0.02040
CJ	0.01944	0.04385	0.13398	0.04147
Cookie	0.15410	0.09461	0.07134	0.17968
Copper	0.16785	0.13888	0.07505	0.25289
Flight	0.37307	0.26871	0.10178	0.04122
LB	0.11911	0.12408	0.10459	0.06381
LeRoy	0.04608	0.03597	0.09590	0.08225
Nellie	0.10003	0.02980	0.08664	0.10178
Red	0.12469	0.26148	0.20898	0.11434
Sammie	0.09674	0.03566	0.29449	0.21716

Table 1 Cortisol levels of each horse pre/ post-RL and pre/ post-behavior tests in µg/dL

Cortisol		t	p-value
Pre-RL	Post-RL	1.41	0.152
Pre-test	Post-RL	0.420	0.538
Pre-test	Post-test	0.0378	0.968
Post-test	Post-RL	0.352	0.709

Table 2 Paired t-test relating cortisol levels during behavior tests and RL

There were no significant differences in cortisol found between pre-RL and post RL, pre-test and post-RL, pre-test and post-test, or post-test and post-RL, as found by a paired t-test.

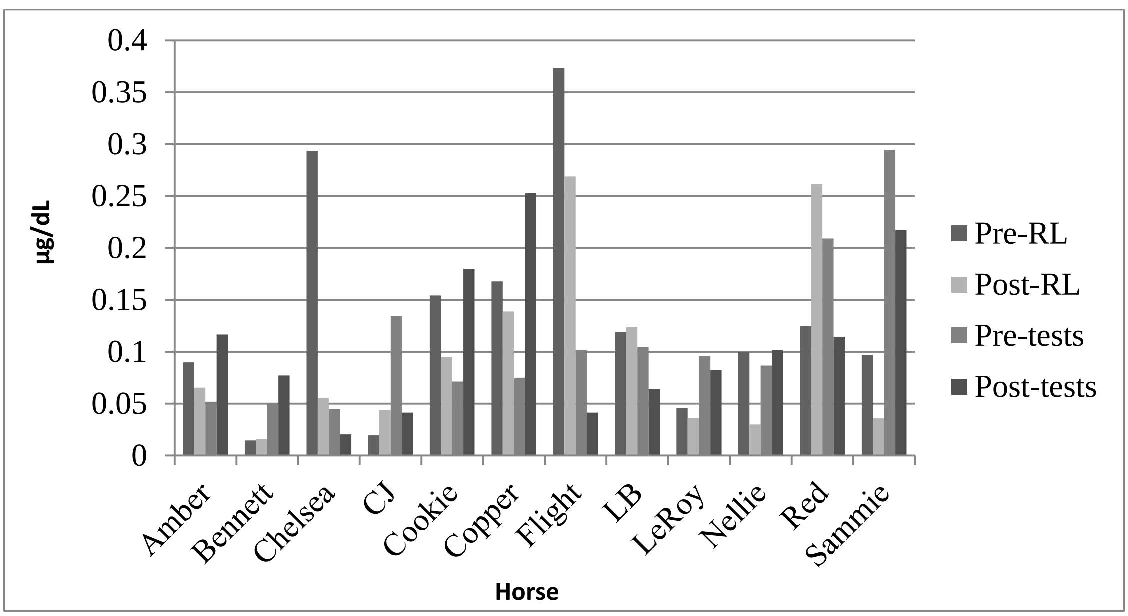

Fig 1 Cortisol level trends for individual horses during behavior tests and RL

Heart Rate Parameters

The following heart rate (HR) variables were quantified: (1) mean HR (bpm) (2) the standard deviation of beat-to-beat intervals (SDRR) and (3) the root mean square of successive beat-to-beat differences (rMSSD, ms). Generally speaking, reductions in the values of HRV indices (SDRR and rMSSD) reflect a shift of the autonomic balance towards a more sympathetic dominance (fight or flight), while increased values of these indices indicate a shift towards a more parasympathetic dominance (Figs. 3, 4). The SDRR estimates the overall HRV and therefore includes the contribution of both branches of the autonomic nervous system to HR variations: It measures the state of balance between sympathetic and parasympathetic activities of the heart. rMSSD focuses on high-frequency, short-term variations of HR, which are mainly due to the activity of the parasympathetic nervous system.

	HRmean (bpm)	**SDRR**	**rMSSD (ms)**
baseline	36.08333(±6.42)	150.12(±66.89)	107.95(±73.38)
arena	63.25(±19.71)	216.14(±103.45)	100.48 (±77.01)
Novel object	57(±16.51)	188.07(±70.73)	83.15(±36.54)
bridge	52.75(±6.14)	199.97(±205.94)	161.02(±249.47)
Riding lesson	65.5(±26.08)	320.33 (±84.86)	103.81(±49.60)

Table 3 Average BPM, SDRR, rMSSD with associated standard deviations

mean HR		**t**	**p- value**
baseline	arena	-4.463	0.001
baseline	novel object	-4.327	0.001
baseline	bridge test	-5.109	0.00004
baseline	riding lesson	-3.565	0.004
arena	riding lesson	-0.2308	0.8
novel object	riding lesson	-0.8626	0.4
bridge test	riding lesson	-1.602	0.1
SDRR		**t**	**p- value**
baseline	arena	-2.405	0.03
baseline	novel object	-3.022	0.01
baseline	bridge test	-1.232	0.2
baseline	riding lesson	-1.723	0.0000007
arena	riding lesson	-2.443	0.03
novel object	riding lesson	-4.192	0.002
bridge test	riding lesson	-1.723	0.1
rMSSD		**t**	**p- value**
baseline	arena	1.033	0.3
baseline	novel object	0.493	0.6
baseline	bridge test	-1.189	0.3
baseline	riding lesson	-0.203	0.8
arena	riding lesson	-1.538	0.2
novel object	riding lesson	-0.702	0.5
bridge test	riding lesson	1.245	0.2

Table 4 Paired t-test relating HR parameters between baseline, behavior tests, and RL

Every horse had an increase in mean HR between baseline and behavioral tests/riding lessons, yet the general trend for the majority of horses was the greatest increase occurring between the baseline and arena test (AR), then decreasing after both the novel object test (NO) and bridge test (BR) (Fig. 2). The mean values of HR, SDRR, and rMSSD were determined for baseline, AR, NO, BR, and RL, respectively (Table 3). Significant increases of HR, determined using a paired t-test and Bonferroni correction, were found between baseline and AR, NO, BR, and RL, whereas significant differences of SDRR were found between baseline and NO, AR, RL, and also between AR and RL, and NO and RL. No significant differences were found with rMSSD (Table 4).

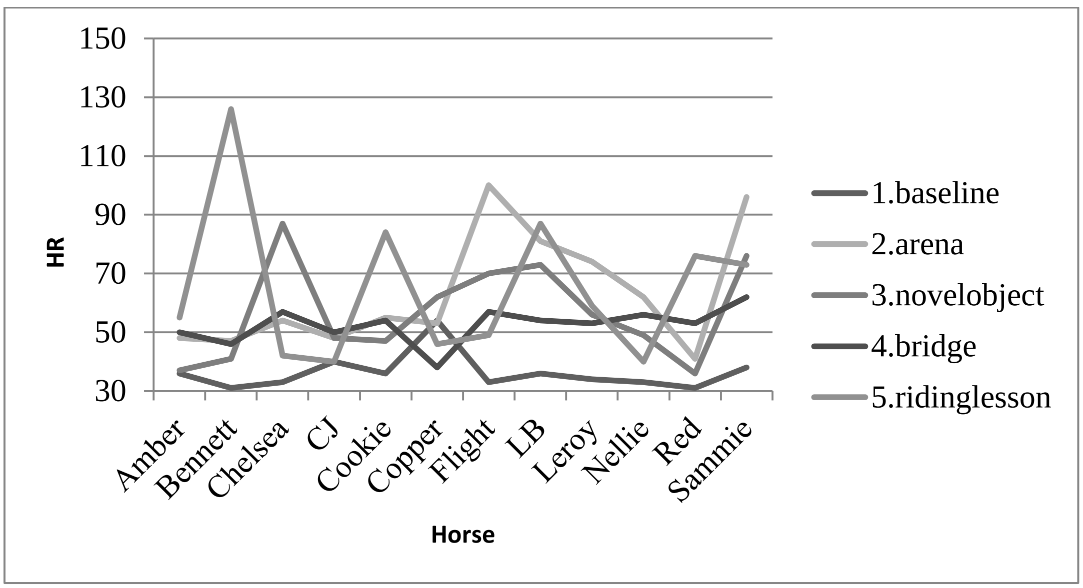

Fig 2 Individual HR values for baseline, AR, NO, BR, and RL

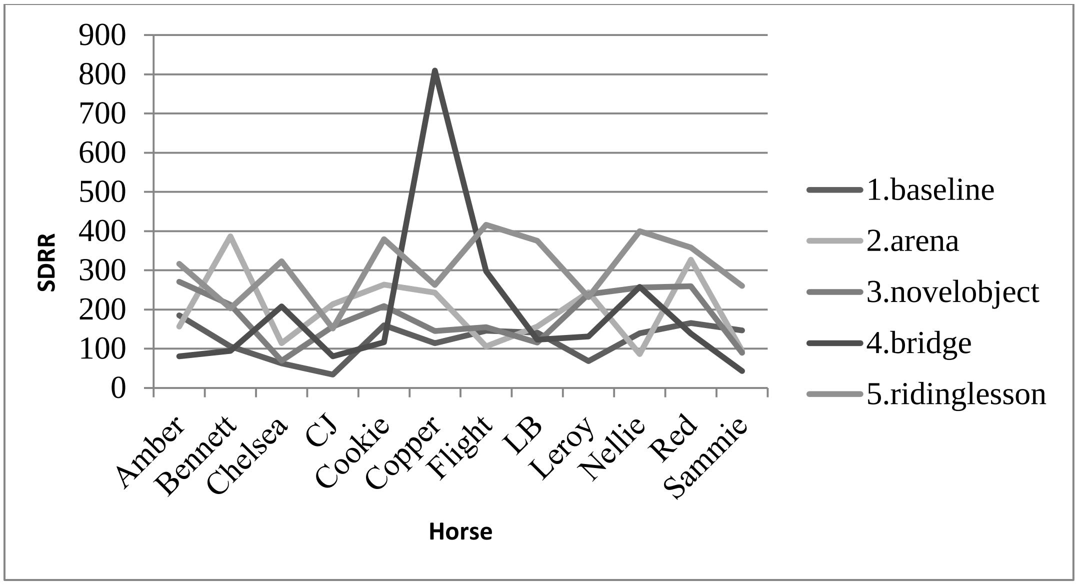

Fig 3 Individual SDRR values for baseline, AR, NO, BR, and RL

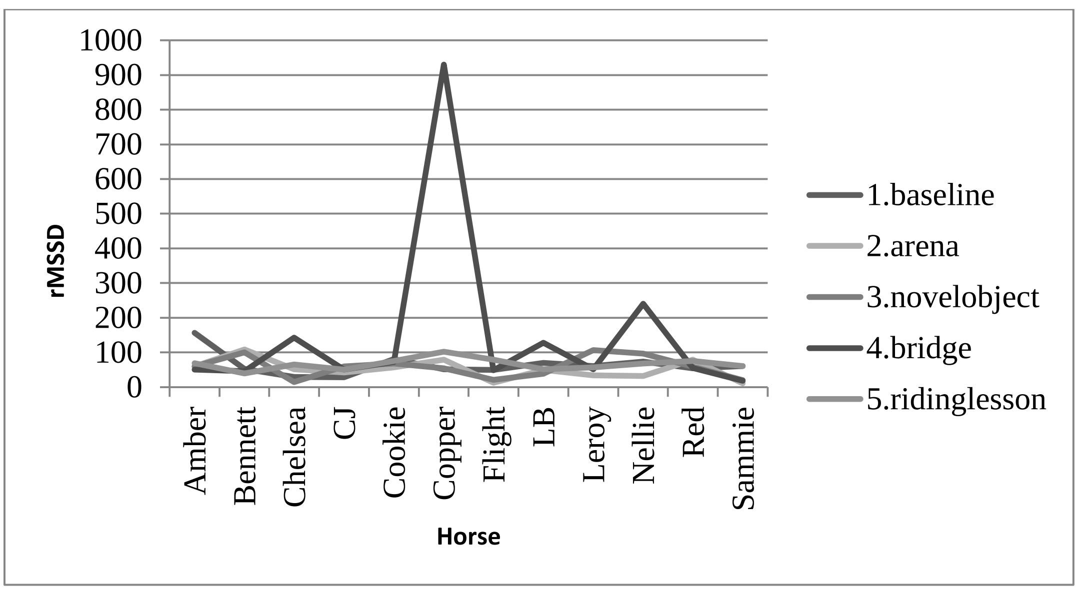

Fig 4 Individual rMSSD values for baseline, AR, NO, BR, and RL

Behavior Tests

The three behavior tests (AR, NO, BR) were designed to assess separation anxiety and fearfulness in the horses; ethograms outlining proven signs of stress and relaxation were constructed for each test, then the average time budgets and number of incidents of signs of stress were recorded (Tables 5, 6, 7).

	canter	**trot**	**Rel_ walk**	**Vig_ walk**	**stand**	**stand_alert**
AR	2.08	30.5	63.19	39.78	31.33	22.89
NO	2.33	13.08	56.58	36.11	25.53	26.97
	stand_ gate	**stand_ people**	**paw**	**graze**	**t_inner_ circle__NO**	
AR	39.11	13.14	1.39	24.58	N/A	
NO	83.44	9.97	1.17	13.4	27.08	

Table 5 Average time budgets of all horses in percent during the AR/NO tests

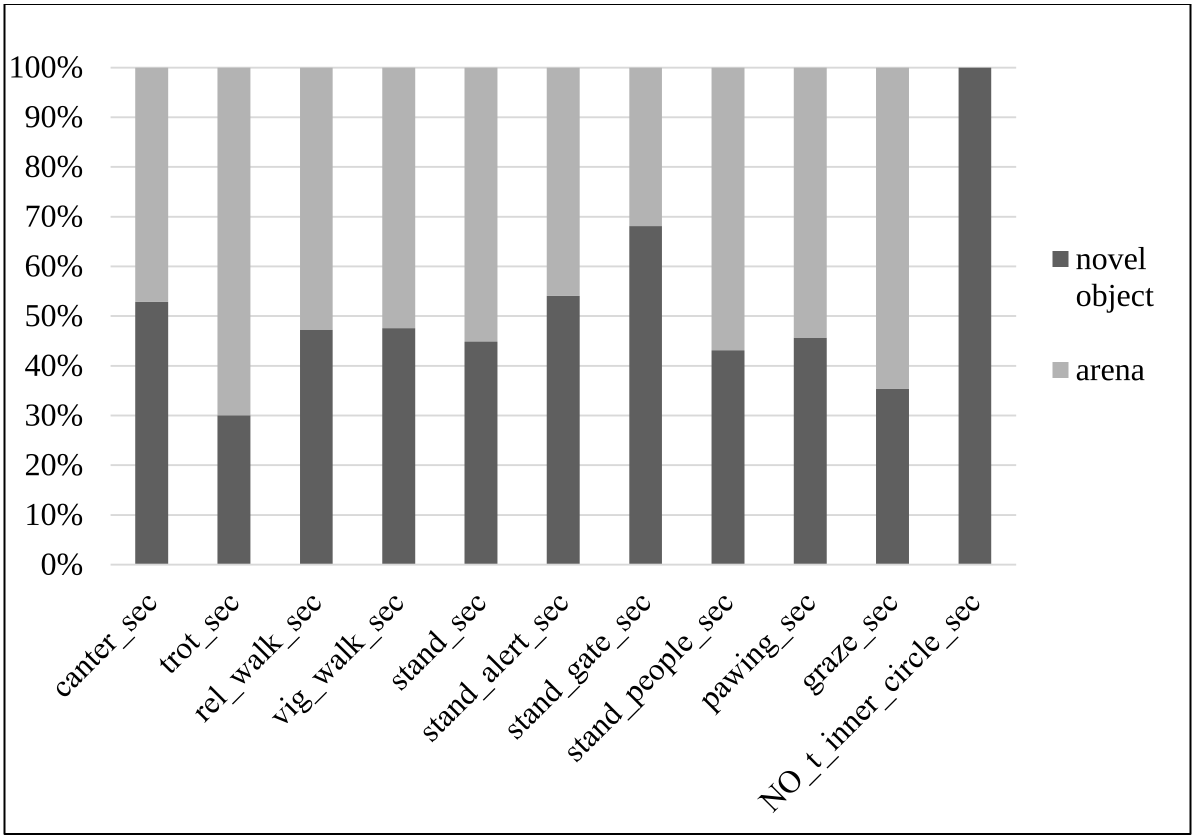

Fig 5 Average time budgets of all horses during the AT/ NO tests

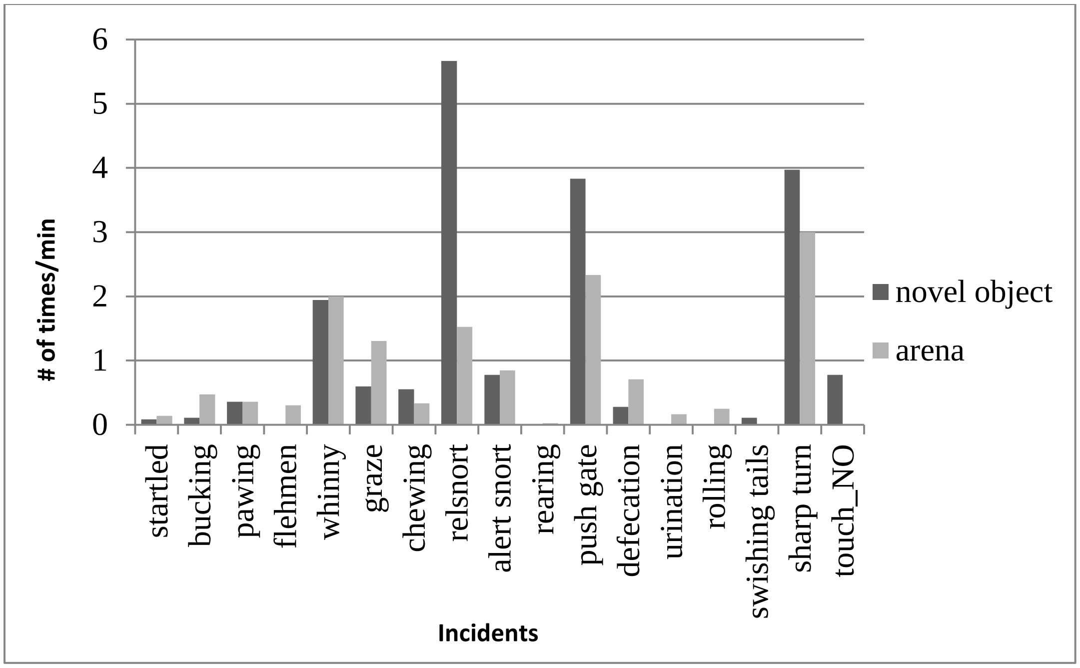

Fig 6 Average incidents per minute for all horses during the AR/ NO tests

	on_bridge (y/n=1/0)	#attempts_bridge	stop@approach #	stand_3sec_on_bridge (y/n=1/0)
BR	0.92	1.22	0.39	0.92

Table 6 Average total number of incidents for all horses during BR tests

Individual riding lessons (RL), everyday work for the horses, were observed and a separate ethogram constructed in order to observe number of incidents indicative of stress (Table 7).

	tail swish	ears back	defecation	head toss	head shake	head turn	lower/extend head
RL	3.5	1.4	0	4	1.5	3	1

Table 7 Average incidents per minute for all horses during riding lessons

On average, more signs of stress were observed during the AR than the NO, BR, or RL, and a significant decrease in observed signs of stress were observed in the RL versus the behavior tests.

		SDRR	**rMSSD**	**BPM**	**Cortisol**
		medium filter	medium filter		
SDRR	medium filter	1			
rMSSD	medium filter	0.719** (<0.001)	1		
BPM		-0.331* (0.021)	-0.493** (< 0.001)	1	
Cortisol		-0.138 (0.350)	0.034 (0.820)	-0.280 (0.054)	1
Behaviors		-0.523** (< 0.001)	-0.504** (< 0.001)	0.022 (0.880)	0.417 (0.318)

**Correlation is significant at the 0.01 alpha
*Correlation is significant at the 0.05 alpha

Table 8 Correlation between HR parameters, BPM, cortisol, and behaviors as defined by Pearson correlation test and p-values

There was no significance between BPM and behavior (r-value of 0.022, p-value of 0.880), BPM and cortisol (r-value of -0.280 and p-value of 0.054), SDRR and cortisol (r-value of -0.138 and p-value of 0.350), rMSSD and cortisol (r-value of 0.034, p-value of 0.820), or cortisol and behavior (r-value of 0.147, p-value of 0.318). There was significance between SDRR and behavior (r-value of -0.523, p-value of < 0.001), SDRR and rMSSD (r-value of 0.719 and p-value of 3.29×10^{-14}), BPM and SDRR (r-value of -0.331 and p-value of 0.021), BPM and rMSSD (r-value of -0.493 and p-value of < 0.001), behaviors and SDRR (r-value of -0.523 and p-value of < 0.001), and behaviors and rMSSD (r-value of -0.504 and p-value of < 0.001).

CHAPTER 5

DISCUSSION

Cortisol

Salivary cortisol samples were obtained from each horse before and after behavior tests and riding lessons; averages of 3.676 (± 2.930) nmol/L and 3.035 (± 2.024) nmol/L were obtained for baseline cortisol values of the riding lessons and behavior tests, respectively. Comparatively, similar studies[26,29] obtained baseline salivary cortisol levels of 2.76 nmol/L and 1.01 nmol/L, respectively. The differences between baseline cortisol levels could be explained by differing methods of salivary collection—in our study, we collected samples by inserting Salimetrics® children's swabs under the horse's tongue for approximately 30 seconds, whereas in the aforementioned studies, samples were obtained by clamping a cotton swab to the horse's tongue. There could be potential risk in such an uncomfortable procedure for the horse—if a clamp is placed on the tongue for 30–40 seconds, the horses may perceive this as uncomfortable, which could influence cortisol values. Another factor that could contribute to a difference in baseline cortisol values across studies are the horses themselves; our statistical analysis did find that some independent variables will affect how stressed a horse will become, such as age and years owned. Thus, depending on the age and background, and potentially other independent variables, such as health conditions or endocrine disorders, variance in cortisol levels will occur. Conclusively, while baseline cortisol values vary greatly across studies, this can be expected considering certain independent variables and methods of collection.

While cortisol levels have been proven by various authors to be an effective physiological measure of stress in horses[17,26] our statistical analysis determined that our cortisol values did not change significantly (all p-values > 0.05) in relation to behavior or HR parameters. There are several possible reasons: (a) time of day, (b) cross-contamination of samples, (c) inconsistent "before" and "after" sampling times, and (d) number of times samples were collected for each horse. (a) Distinct circadian rhythms of cortisol levels have been determined for horses.[5] A peak occurs in the morning and continues to decrease throughout the day. This rhythm can influence the obtained cortisol levels such that if salivary samples are obtained in the morning, it would be unknown whether higher levels are due to stress alone or because of the natural peak. Further, since afternoon cortisol levels are lower, the values for the riding lessons would overall be lower compared to the values for the behavior tests, which were obtained in late morning. (b) Cross-contamination likely occurred due to our method of sampling. Gloves were not consistently disposed of between sampling horses. Some contamination could have occurred due to saliva already present on the gloves. (c) We obtained baseline samples for each horse at approximately the same time before behavior tests which were performed consecutively in groups of 6 horses per day on two consecutive days. Horses were subjected to the behavior tests individually and in sequence. Allowing some horses to graze while waiting for their turn to be tested while others were subjected to the tests could have potentially influenced cortisol levels. (d) Cortisol samples should be obtained

15–20 minutes after the introduction of a potential stressor. While the saliva samples after the tests were obtained right after the last test (bridge test), the interval could have been too long to observe peak levels of cortisol, which occur approximately every 20 minutes.[16] Other studies sampled every 10-15 min,[26,29] and it would have been advantageous to obtain a saliva sample after each test. This would have provided data taken approximately every seven to ten minutes.

Heart Rate Parameters

BPM

The average baseline values recorded are within the normal range for adult horses "at rest," between 28–44 BPM. According to similar studies,[30,37] average BPM values increased significantly between baseline values after horses were subjected to a stressor. HR alone is not considered to be an accurate physiological measure of stress due to the factors that can alter a horse's heart beat; naturally, when an animal is moving, exercising, or in pain, HR will elevate. Tests designed for stress that involve fair amounts of movement or exercise cannot assume that the elevation in HR is due to stress alone. Our analyses show that an increase in HR is significant ($p<0.05$) between the baseline values and all behavior tests, as well as the riding lesson. Horses moved not at all, slowly, or faster during the arena and novel object tests at will. During the bridge test and the riding lessons, horses were mostly walking, with short trot episodes during the riding lessons. The elevated heart rate during the behavior tests can be primarily related to separation anxiety and fearfulness, which tempted the horses to move faster, exhibit sharp turns, display bucking, trot and canter episodes, which in turn elevated the horse's heart rates.

SDRR

Average values are concurrent with similar studies,[29,37] although in these studies, over the course of the tests designed for stress, overall SDRR values decreased, while between baseline and tests/ RL our SDRR values increased. SDRR is a measure of the variability across every RR interval for each horse, so the increases and overall larger values of SDRR in our study reflect that our test subjects are under more vagal dominance than horses utilized in similar studies. The results indicate a higher sympathetic dominance, and therefore possibly higher stress levels, during the behavior tests. Higher SDRR values during the riding lessons indicate a wider spectrum of autonomous nervous system responses, and a possibly lower stress level that horses experienced during the riding lessons. Further, this is an indication that New Beginnings Therapeutic Riding has chosen suitable horses for this type of work.

rMSSD

rMSSD is the root mean square of successive RR intervals (heart beats), and thusly is the primary variable used to determine variations that represent changes from parasympathetic nervous system (rest and digest) to sympathetic nervous system control (fight or flight, stress). The higher rMSSD, the more vagal influence is evident. Our baseline values are within previously documented range. Comparable studies[30,37] documented values of approximately 74.5 (±8.81) msec and 46.3 (±4.8) msec, respectively. Younger horses are more excitable and exhibit higher heart rates readily,[24] which would explain lower rMSSD values reported by Visser et al.[37] In our study, only one horse was younger than 10 years, the majority of horses were middle aged, and three horses were twenty-one years and older. Other factors that have an influence on vagal dominance are type and breed of horse. The therapeutic riding horses in our study were mostly Quarter Horses. One Thoroughbred, age five, actually displayed the highest heart rate values during the behavior tests and the riding lessons. There was an overall increase in rMSSD values during the bridge test compared to the arena test, the novel object test, and the riding lesson. The horses in our study are used on a daily basis for therapeutic riding, and are accustomed to being handled frequently by many different people with various horse experience. Disabled riders, as well as their horses, are exposed to various objects and handling situations regularly. The bridge test, also known as the novel handling test, determines how a horse will react to a novel object when being accompanied by a handler. A therapeutic riding horse is more accustomed to follow the handler, whereas horses in the arena- and novel object tests are separated from the herd, and subjected to a novel object. While our baseline values are within normal ranges for horses, the significant decrease of rMSSD between baseline values and tests/ RL in our study match a similar trend within the aforementioned studies, indicating higher stress levels during behavior tests and riding lessons.

Behavior

Ethograms were developed based on previously reported observations[35,36] that have been used extensively by other researchers in the development of their analysis of equine stress behaviors. Our analyses show significance changes ($p<0.05$) between HR parameters BPM, SDRR, rMSSD and behaviors (Table 10). There was slight increase of stress-related behaviors (vigilant walk, trot, whinny, startled, alert snort, defecation) in the AR versus the NO, which could be due to a low degree of habituation. Horses were subjected to the novel object test after five minutes in the arena, and may have been more comfortable the second time around. Horses were less disturbed by unfamiliar objects in their immediate environment than during separation. This is a positive indication for the riding center regarding suitability of horses for their work. Therapeutic riding horses regularly come into contact with unfamiliar items in their everyday work.

Conclusion

The primary objective of this study was to determine whether objective measures of physiological parameters and behavior analysis were practical and accurate ways to assess emotionality of horses used in therapeutic programs; our goals were to define more stringent methods of determining suitability of horses for therapeutic horseback riding, help create a more safe and fun experience for riders involved in therapeutic riding programs, and to enhance equine welfare by possibly decreasing equine wastage by assessing what horses can handle stress-wise for this type of work. Although other studies describe changes in cortisol as a useful measure of stress in horses, it is impractical for assessing horses suitable for a therapeutic riding facility. Range of cortisol values depends on multiple factors, such as time of day of sampling, sampling intervals, horse health, sample storage, and laboratory techniques. Furthermore, sampling equipment, storage tubes, assays or laboratory fees are financially restrictive for a facility relying on donations.

Based on aforementioned findings, however, we have determined that heart rate variability parameters are reliable indicators of equine emotionality and stress. Our statistical analyses showed significant changes ($p<0.05$) between HR parameters and observed behaviors in the behavior tests and the riding lesson. Baseline values significantly increased during all tests and riding lessons. Other studies have shown the validity of HRV as stress indicators as well.[29,38,37] Equine heart rate monitors are fairly inexpensive as a one-time purchase. Employees or volunteers can be trained to use a heart rate monitor and apply it to specific situations with their horses. This method of stress evaluation could be a simple, inexpensive and practical method for objectively determining the emotionality and suitability of potential therapeutic riding horses. Similarly, ethograms constructed with similar parameters to those in this study administered and completed by an objective third-party at least somewhat trained in equine behavior is a practical, affordable, and valid assessment of horse emotionality and suitability, as proven by our findings of significance ($p<0.05$) between HR parameters and behaviors.

There was a significant decrease of observed stress behaviors during the riding lessons compared to the behavior tests. Well-trained and regularly used horses overcome eventual higher degrees of emotionality through training and habituation. When horses were subjected to the behavior tests, more stress-related behaviors were observed. This provides evidence that individual horses are not as impervious to agitation as a riding lesson would indicate. Therefore, it is important that similar objective tests be conducted for each horse being tested for therapeutic riding purposes. In temperamental horses, signs of stress will become apparent sooner or later, and a dangerous and possibly deadly outcome can be avoided by determining horses' receptiveness to specific situations, as shown in the behavior tests. This study indicates that an objective assessment of horses for therapeutic riding purposes is best achieved through routine testing of horses in behavior tests, supported with heart rate parameter evaluation.

REFERENCES

[1]Alexander, S. L., & Irvine, C. H. G. (1998). The effect of social stress on adrenal axis activity in horses: the importance of monitoring corticosteroid-binding globulin capacity. *Journal of Endocrinology, 157*(3), 425–432. http://dx.doi.org/10.1677/joe.0.1570425

[2]Alvares, G. A., Quintana, D. S., Kemp, A. H., Van Zwieten, A., Balleine, B. W., Hickie, I. B., & Guastella, A. J. (2013). Reduced Heart Rate Variability in Social Anxiety Disorder: Associations with Gender and Symptom Severity. *PloS ONE, 8*(7), 1–9. http://dx.doi.org/10.1371/journal.pone.0070468

[3]Anderson, M. K., Friend, T. H., Evans, J. W., & Bushong, D. M. (1999). Behavioral assessment of horses in therapeutic riding programs. *Applied Animal Behaviour Science, 63*(1), 11–24. http://dx.doi.org/10.1016/s0168-1591(98)00237-8

[4]Bachmann, I., Bernasconi, P., Herrmann, R., Weishaupt, M. A., Stauffacher, M. (2003). Behavioral and physiological responses to an acute stressor in crib-biting and control horses. *Applied Animal Behaviour Science*, *82*(4,5), 297–311. http://dx.doi.org/10.1016/s0168-1591(03)00086-8

[5]Becker-Birck, M., Schmidt, A., Lasarzik, J., Aurich, J., Möstl, E., Aurich, C. (2013). Cortisol release and heart rate variability in sport horses participating in equestrian competitions. *Journal of Veterinary Behavior*, *8*, 87–94. http://www.sciencedirect.com/science/article/pii/S1558787812000743

[6]Benda, W., McGibbon, N.H., & Grant, K.L. (2003). Improvements in Muscle Symmetry in Children with Cerebral Palsy After Equine-Assisted Therapy (Hippotherapy). *Journal of Alternative & Complementary Medicine, 9*(6), 817–825. http://dx.doi.org/10.1089/107555303771952163

[7]Bertoti, D. B. (1988). Effect of Therapeutic Horseback Riding on Posture in Children with Cerebral Palsy. *Physical Therapy*, *68*(10), 1505–1512. Retrieved from http://www.physther.net/content/68/10/1505.full.pdf

[8]Bream, J. A., & Spangler, W. Q., Jr. (n.d.). Therapeutic Horseback Riding: an Overview. Retrieved from: http://www.catra.net/info/overview.html

[9]Corring, D., Lundberg, E., & Rudnick, A. (2013). Therapeutic Horseback Riding for ACT Patients with Schizophrenia. *Community Mental Health Journal, 49*(1), 121–126. http://dx.doi.org/10.1007/s10597-011-9457-y

[10]David, C. (2007). Horse Play. (cover story). *Parks & Recreation*, *42*(10), 34–38. Retrieved from http://chelandavid.com/parks.pdf

[11]Dingman, A. (2008). Hoof Prints: Equine Therapy for Autistic Children. *Encounter: Education for Meaning and Social Justice*, *21*(4), 11–13. Retrieved from http://great-ideas.org/Rhombus/Encounter214(Winter2008).pdf

[12]Donating Your Horse to a PATH Intl.. Therapeutic Riding Center. (n.d.). Retrieved from http://www.pathintl.org/images/pdf/about-narha/PATH-Intl-Donating-Your-Horse.pdf

[13]Drnach, M., O'Brien, P. A., & Kreger, A. (2010). The Effects of a 5-Week Therapeutic Horseback Riding Program on Gross Motor Function in a Child with Cerebral Palsy: A Case Study. *Journal of Alternative & Complementary Medicine, 16*(9), 1003–1006. http://dx.doi.org/10.1089/acm.2010.0043

[14]Hart, J. (2013). Association between heart rate variability and manual pulse rate. *Journal of the Canadian Chiropractic Association*, *57*(3), 243–250. Retrieved from http://cdn.agilitycms.com/jcca/PDF/2013/2013-3/JCCA-v57-p243-3g-Hart.pdf

[15]History of Therapeutic Riding/Equine Assisted Activities. (n.d.). Retrieved from http://www.stablelifeinc.org/historyEAT.html

[16]J. Jewell, personal communication, April 24, 2013.

[17]Kedzierski, W., Strzelec, K., Cywinska, A., & Kowalik, S. (2013). Salivary Cortisol Concentration in Exercised Thoroughbred Horses. *Journal of Equine Veterinary Science, 33*(12), 1106–1109. http://dx.doi.org/10.1016/j.jevs.2013.04.011

[18]König von Borstel, U., Euent, S., Graf, P., Konig, S., Gauly, M. (2011). Equine behaviour and heart rate in temperament tests with or without rider or handler. *Physiology & Behavior, 104*(3), 454–463. http://dx.doi.org/10.1016/j.physbeh.2011.05.010

[19]Lai, F., Chang, W., & Jeng, C. (2012). The relationship between physical activity and heart rate variability in orthotopic heart transplant recipients. *Journal of Clinical Nursing, 21(*21/22), 3235–3243. http://dx.doi.org/10.1111/j.1365-2702.2012.04070.x

[20]Lanning, B. A., & Krenek, N. (2013). Guest Editorial: Examining effects of equine-assisted activities to help combat veterans improve quality of life. *Journal of Rehabilitation Research & Development, 50*(8), vii–xiii. http://dx.doi.org/10.1682/jrrd.2013.07.0159

[21]Liu, J., Wei, W., Kuang, H., Zhao, F., & Tsien, J. Z. (2013). Changes in Heart Rate Variability Are Associated with Expression of Short-Term and Long-Term Contextual and Cued Fear Memories. *PloS ONE, 8*(5), 1–14. http://dx.doi.org/10.1371/journal.pone.0063590

[22]Lloyd, A. S., Martin, J. E., Bornett-Gauci, H. L. I., & Wilkinson, R. G. (2007). Evaluation of a novel method of horse personality assessment: Rater-agreement and links to behaviour. *Applied Animal Behaviour Science, 105*, 205–222. http://dx.doi.org/10.1016/j.applanim.2006.05.017

[23]Momozawa, Y., Ono, T., Sato, F., Kikusui, T., Takeuchi, Y., Mori, Y., & Kusonose, R. (2003). Assessment of equine temperament by a questionnaire survey to caretakers and evaluation of its reliability by simultaneous behavior test. *Applied Animal Behaviour Science, 84*(2), 127–138. http://dx.doi.org/10.1016/j.applanim.2003.08.001

[24]Normal Horse Vital Signs and Health Indicators. In *thehorse.com*. Retrieved from http://www.thehorse.com/articles/31854/normal-horse-vitals-signs-and-health-indicators

[25]Nunez, C. M. V., Adelman, J. S., Smith, J., Gesquiere, L. R., & Rubenstein, D. I. (2014). Linking social environment and stress physiology in feral mares (*Equus caballus)*: Group transfers elevate fecal cortisol levels. *General and Comparative Endocrinology, 196,* 26–33. http://dx.doi.org/10.1016/j.ygcen.2013.11.012

[26]Peeters, M., Sulon, J., Beckers, J-F., Ledoux, D., Vandenheede, M. (2011). Comparison between blood serum and salivary cortisol concentrations in horses using an adrenocorticotropic hormone challenge. *Equine Veterinary Journal, 43*(4), 487–493. http://dx.doi.org/10.1111/j.2042-3306.2010.00294.x

[27]Reed, M. J., Robertson, C. E., & Addison, P. S. (2005). Heart rate variability measurements and the prediction of ventricular arrhythmias. *Quarterly Journal of Medicine, 98*(2), 87–95. http://dx.doi.org/10.1093/qjmed/hci018

[28]Rotterman, S. (2010). Jubilee, A Post-War Dressage Hero. Retrieved from http://www.eurodressage.com/equestrian/2010/10/22/jubilee-post-war-dressage-hero

[29]Schmidt, A., Aurich, J., Mostl, E., Muller, J., & Aurich, C. (2010a). Changes in cortisol release and heart rate and heart rate variability during the initial training of 3-year-old sport horses. *Hormones and Behavior, 58*(4), 628–636. http://dx.doi.org/10.1016/j.yhbeh.2010.06.011

[30]Schmidt, A., Mostl, E., Wehnert, C., Aurich, J., Muller, J., & Aurich, C. (2010b). Cortisol release and heart rate variability in horses during road transport. *Hormones and Behavior, 57*(2), 209–215. http://dx.doi.org/10.1016/j.yhbeh.2009.11.003

[31]Silver, J.R. (2002). Spinal injuries resulting from horse riding accidents. *Spinal Cord, 40*(6), 264–271. http://dx.doi.org/10.1038/sj.sc.3101280

[32]Smith-Osborne, A., & Selby, A. (2010). Implications of the Literature on Equine-Assisted Activities for Use as a Complementary Intervention in Social Work

Practice with Children and Adolescents. *Child & Adolescent Social Work Journal, 27*(4), 291–307. http://dx.doi.org/10.1007/s10560-010-0201-1

[33]Strickland, C. (2000). Equine-Related Human Injuries. *The Horse: Your Guide to Equine Health Care, 17*(10), 61–66. Retrieved from http://www.thehorse.com/articles/10102/equine-related-human-injuries

[34]Violette, K., & Wilmarth, M. A. (2009). Hippotherapy: A Therapeutic Treatment Strategy. Retrieved from http://gallopnyc.org/resources/research/hippotherapy-art-violette/

[35]Visser, E. K., van Reenen, C. G., Engel, B., Schilder, M. B. H., Barneveld, A., & Blokhuis, H. J. (2003a). The association between performance in show-jumping and personality traits earlier in life. *Applied Animal Behaviour Science, 82*(4), 279–295. http://dx.doi.org/10.1016/s0168-1591(03)00083-2

[36]Visser, E. K., van Reenen, C. G., Rundgren, M., Zetterqvist, M., Morgan, K., & Blokhuis H. J. (2003b). Responses of horses in behavioural tests correlate with temperament assessed by riders. *Equine Veterinary Journal, 35*(2), 176–183. http://dx.doi.org/10.2746/042516403776114108

[37]Visser, E. K., van Reenen, C. G., van der werf, J. T. N., Schilder, M. B. H., Knaap, J. H., Barneveld, A., & Blokhuis, H. J. (2002). Heart rate and heart rate variability during a novel object test and a handling test in young horses. *Physiology & Behavior, 76*(2), 289–296. http://dx.doi.org/10.1016/s0031-9384(02)00698-4

[38]von Borell, E., Langbein, J., Despres, G., Hansen, S., Leterrier, C., Marchant-Forde, J., Marchant-Forde, R., Minero, M., Mohr, E., Prunier, A., Valance, D., & Veissier, I. (2007). Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—A review. *Physiology & Behavior, 92*(3), 293–316. http://dx.doi.org/10.1016/j.physbeh.2007.01.007