2017

SERUM CORTISOL CONCENTRATIONS AND BEHAVIOR ASSESSMENT AS TOOLS FOR EVALUATING STRESS IN HORSES USED IN THERAPEUTIC OR UNIVERSITY RIDING PROGRAMS

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A Thesis
Presented to
the Faculty of the Hutson School of Agriculture
Murray State University
Murray, Kentucky

In Partial Fulfillment
of the Requirements for the Degree
of Master of Science in Agriculture

by Shikun Chen
December 2017

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Acknowledgements

I would first like to express my special appreciation and thanks to my advisor Professor Dr. Shea Porr, she has been a tremendous mentor for me. I would like to thank her for bringing me to equine science and encouraging my research. She steered me in the right direction whenever I needed it. She always given the best suggestion whenever I ran into a trouble spot or had a question about my research or writing.

I would especially like to thank Dr. Amanda Davis and Monique Hovey who were involved in this research project. Without their help and input, the experiments could not have been successfully conducted.

Finally, I must thank my parents for standing behind me in all that I do. This accomplishment would not have been possible without them.

Abstract

Stress is known to have a negative impact on the health and well-being of animals. Physiological and behavioral changes offer objective and easy to use methods of evaluating stress in horses. However, there are limited studies showing a relationship between changes in stress-related behavior and stress-related serum cortisol concentrations in horses. This study evaluated the relationship between stress-related behavior and cortisol concentration changes in horses used in a university equine program and a therapeutic riding program. Behavior was evaluated by two trained observers during multiple riding sessions. Audio recordings were made during direct observation, and video recordings were used to later confirm audio observations. Serum samples were collected before, immediately after, and 30 minutes after riding, and were evaluated for cortisol concentrations. Results showed no change in behavior scores in either group of horses, but serum cortisol concentrations increased in university riding horses as the semester progressed. However, all serum cortisol concentrations remained within or below normal ranges. A relationship between stress related behavior and cortisol concentration changes was not shown clearly, suggesting horses were in a low-stress environment.

Keyword: serum cortisol, horse behavior, stress, university riding program, therapeutic riding program

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Introduction

Stress has been defined as the biological response elicited when an individual perceives a threat to its homeostasis (Moberg, 2000). Animals experiencing stress may display abnormal behaviors (Kaiser et al., 2006), excessive hormone release (Turner, 2014), decreases in performance (Alexander and Irvine, 2001), or various health problems (Mills and Nankervis, 1999; Ayala et al., 2012). Alterations in these variables could be assessed in order to evaluate stress levels in horses. Hormone changes have been a widely accepted and accurate method commonly used to monitor stress. The relationship between stress levels and changes in cortisol concentrations has been verified (Matteri et al., 2000). Observing body language provides another oft-used method, but there is no standard method of evaluating behavior that is universally accepted (Kaiser et al., 2006; Young et al., 2012; Collins et al., 2017). This method also requires a more experienced observer and may not be as accurate, but studies have shown a relationship between stress levels and changes in equine behavior (Seaman et al., 2002). However, a relationship between stress levels and cortisol changes has not been strongly supported.

Throughout history, horses have been managed and used in a variety of ways as needed by human beings (Potter et al., 1994; Anderson et al., 1999; Hall et al., 2013; Colston et al., 2015), and such interactions could lead to stress. If increased stress leads to inappropriate behavior, this could interfere with the horse’s usefulness in programs involving handling and riding. Also, increased stress can lead to decreased health and welfare (Malmkvist et al., 2012; Budzyńska, 2014). Ill horses can cost horse owners both time and money, and such illness is certainly not what most horse owners want for their animals.

The purpose of this review was two-fold: first, to explore stress in horses to better understand causes, symptoms, and management of stress; and second, to evaluate the use of horses in today's society. The objective of the subsequent research was to evaluate the relationship between changes in behavior and cortisol concentration changes in horses used in either a university equine program or a therapeutic riding program. The hypothesis was that horses that were experiencing more stress would exhibit more negative behaviors and show higher serum cortisol concentrations than less-stressed counterparts.

Review of the Literature

Use of Animals

Humans have a long history of beneficial relationships with animals. As human civilization developed, man's knowledge and understanding of animals became more comprehensive, and the relationship between humans and animals underwent tremendous changes. Between 4000 and 3000 B.C., humans began to domesticate the horse on the steppes north of the Black Sea (Parker, 2012). Before domestication, horses were mainly hunted for food. After domestication, the horse was more often used in agriculture, war, and transportation. In the 1900's, the invention of the automobile and the tractor replaced the horse much of these areas. The uses of horses in today's society is changing. Since equestrianism appeared at summer Olympic Games in 1900 (IEF, 2017), horses were more frequently trained to be ridden in a variety of competitions, from racing to show jumping to western pleasure. Today, horses are used in a variety of new ways, including competitions, recreation, therapeutic riding (Potter et al., 1994; Anderson et al., 1999), and for research and education (Hall et al., 2013; Colston et al., 2015). In the United States, recreational activities accounts for nearly 42% of horse use, while showing and competition accounts for another 30% (AHCF, 2005). In addition, horses are also used in teaching and research in order to add to the body of knowledge not only about equine health care and management, but also serve as a model for other species.

Horse Use in University Programs

With an increasing interest in equine science, more university programs are developing horse-related courses and conducting research in this area. Many programs also offer riding, either in classes or as part of competitive university teams. Riders in university classes may have little or no experience with horses, and this may cause discomfort or stress in the animals. One study reported that incorrect rider posture and instability of the rider may harm the horse and lead to various diseases and disorders of the muscular skeletal system (de Cocq et al., 2009). This could result in a negative effect on the horse's performance in classes, particularly if the horse is used frequently because it is good in beginning classes with novice riders and handlers. Horses can also be used in other ways at a university. Lecture-based classes may use horses for laboratories, research projects may use horses to study a variety of issues, and many programs have equestrian teams that practice and compete with university-owned horses. However, there are few studies reporting data on horse use in university and college programs.

Horse Use in Therapeutic Riding Programs

Within the last 20 years, the horse use of horses for therapeutic riding has attracted attention (Potter et al., 1994; Anderson et al., 1999). These programs use horses as a method of therapy to treat various disabilities in humans. The benefits of equine assisted activities and therapies (EAAT) or therapeutic riding have been shown to affect four categories of disability in people: physical, psychological, functional (cognitive), and educational (Scott, 2005; Sterba, 2007; Bass et al., 2009).

Making a treatment plan according to disability type can maximize the benefit to humans, but the effect on the horse may be overlooked. As more organizations develop

EAAT programs, and particularly given that these programs often use older, more well-trained horses, equine welfare and rider safety becomes a concern. Only a few studies have been conducted on horse behavior and physiological changes during therapeutic riding sessions (Anderson et al., 1999; Kaiser et al., 2006; Fredrickson-MacNamara and Butler, 2010; Turner, 2014).

Potential Problems with Horse Use

Animal-assisted programs often utilize selection procedures intended to reduce risks associated with human-animal contact (Fredrickson-MacNamara and Butler, 2010). For example, dogs may injure people by biting or scratching, and horses may bite or kick (McGreevy and McLean, 2005). One survey showed that there were an estimated 13,400 emergency room visits nationwide for horse-related injuries among children younger than 15 years of age (Jagodzinski and DeMuri, 2005). An estimated 102,904 persons with non-fatal horse related injuries (35.7 per 100,000 population) were treated in American emergency rooms each year from 2001 to 2003. Most patients were injured while mounted on a horse (66.1%), and most commonly either fell or were thrown by the horse (Thomas et al., 2006). Another survey found that 27.5% of riders aged 25 years and younger who rode six or more times a year had had at least one horse related injury experience in the previous two years (Christey et al., 1994). It should be noted that riders are not the only ones who can be injured. In a survey of 216 Swiss veterinarians, 75% had been kicked by a horse at least once a year, and 3% were kicked 5 to 10 times a year (Hausberger et al., 2008). Animals most commonly injuring veterinarians were bovine 46.5%, canine 24.2%, and equine 15.2% (Landercasper et al., 1988).

Regardless whether the injury resulted from being bitten by dogs or kicked by horses, most injuries were the result of an animal's response to stress. Behavioral problems will generally only be treated if they are undesirable to people (Cooper and Mason, 1998), so it is not hard to assume that many accidents relating to riding horses appear to occur when horses become stressed. Therefore, measuring the degree of stress in the animal and managing stress is very important for human and equine safety wellbeing.

Assessing Stress

Animals show signs of stress in many ways; physiological and behavioral changes are common. Physiological measurements may include hormonal changes or heart rate or respiratory changes, while behavioral may relate to fight-or-flight responses. Neither of these methods are perfect, however, and results from research trials sometimes conflict.

The most common way to assess stress is through the analysis of hormones, such as glucocorticoids, catecholamine, prolactin, or adrenocorticotropic hormone (Möstl and Palme, 2002). Salivary or blood cortisol concentrations are well-known indictors used to measure stress. Cortisol is a steroid hormone that is released in response to stress, and changes in cortisol concentrations has proven to be an accurate method to evaluate stress in horses (Peeters et al., 2011; Turner, 2014). By comparing cortisol concentration changes during the progress of a disease in horses, researchers were able to evaluate the relationship between diseases and stress (Ayala et al., 2013). However, horses presented with food to arouse interest showed no change in cortisol concentrations (Bachmann et al., 2003).

Heart rate variability (HRV) is the variation in intervals between heartbeats and is commonly used to assess stress in horses (Mohr et al., 2000; Turner, 2014). For example, HRV was use to assess horses at rest in familiar surroundings and those exposed to mental (environmental change where animals were moved to a new location) and physical stress (exercise on an aqua-treadmill). Results showed horses under greater stress had lower HRV (Mohr et al., 2000). Warmblood horses doing low intensity exercise showed decreased HRV and increased signs of behavioral stress (Rietmann et al., 2004). Using HRV as a physiological response to an acute stressor in crib-biting and control horses, Bachmann et al. (2003) showed that crib-biting horses were more stress sensitive and less psychologically flexible than controls.

Objectively identifying behavioral signs that the horse is either comfortable or uncomfortable with the activity in which it is participating may be one way to measure stress of the ridden horse. Observation of behavioral signs relating to pain, fear, anxiety, and mental state in the ridden horse can help with assessing horses and ensures a mutually positive experience for both horse and human partner (Hall et al., 2013). Head tossing or raising/lowering the head excessively, showing the ears pinned back, and defecation are common stress behaviors observed in ridden horses (Kaiser et al., 2006; Young et al., 2012). More severe or aggressive behavior may include bite or kick (rear leg) threat, actual bites or kicks, strikes (front leg), rearing, or chasing (Seaman et al., 2002). A more detailed description of behaviors is listed in Appendix A.

Causes of Stress in Horses

There are many aspects of the relationship between humans and animals that might have an impact on equine welfare. Rider interaction with the horse, individual horse variability, as well as tack and equipment issues can lead to increased stress. Any or all of them could come into play depending on the circumstances.

Rider-related Factors: The skill or experience level of a rider has the potential to influence stress in horses. Greater rider experience has been shown to change the rider's distribution of pressure on a horse's back (Janura et al., 2009), suggesting that more experienced riders had better balance. A less skilled or experienced rider may be more inclined to ride in an unbalanced fashion, be less specific with cues to the animal, or may transmit their own uncertainty to the horse. Each of these could result in increased equine stress. Also, skilled riders facilitate the phase synchronization between the rider and the horse and exhibit a fluid and flexible motion in their adjustments to the horse's movement, whereas less skilled riders have been shown to be stiff and tense in their adjustments (Lagarde et al., 2005). Skilled riders also kept their pelvis closer to the mid-position and further forward whereas less skilled riders tilted their pelvis further to the right and more backwards (Münz et al., 2013). Persistent crookedness of a rider could potentially, by physical or behavioral influence, cause asymmetry in horse locomotion, resulting in an established asymmetric locomotors pattern and/or secondary pain (Greve and Dyson, 2013). Both of them may cause stress in horses. That being said, there is very little research showing that a rider's skill or experience directly causes stress in horses. An evaluation of changes in cortisol concentration in horses ridden by riders with various levels of experience showed that experience level of the rider had no effect on equine responses (Ille et al., 2013). Finally, rider gender has also been shown to have no effect on equine performance or stress responses (Ille et al., 2014).

Horse-related Factors: Intrinsic horse behavior may be a factor in how much stress an animal experiences. More nervous horses may be more susceptible to stress than calmer ones. Temperament trait of horses is therefore a key factor to be considered when placing horses into a program. A survey of recreational horse owners as well as equine professionals showed that horses with a more balanced temperament were favored because it simplified daily work with horses, improved the relationship between the horse and human, and made for more comfortable and safe handling (Graf et al., 2013).

Tack and Equipment Factors: Back pain in horses can be caused by poorly fitting equipment, particularly saddles (Harman, 1999). Pain can also increase stress in an animal. Changes in weight distribution of the rider and application of forces by the rider's legs during riding directly results in a changed normal force distribution underneath the saddle (De Cocq et al., 2010). Back movement was measured in horses that were unloaded, wearing a girth, wearing a saddle only, and wearing a saddle with weight. Both the saddle only and weighted conditions were shown to have a significant influence on back kinematics, suggesting that rider weight could have an impact on equine back health (De Cocq et al., 2004). Another review provided an analysis as to how the interaction between horse-related factors, rider-related factors, and saddle-related factors related to equine performance. Specifically, rider errors or ill-fitting equipment resulted in poor performance by the horse (Greve et al., 2013).

Other Factors Affecting Equine Stress

Equine behavior, riders, tack and equipment are directly related to the use of horses in many programs. In addition to these direct effects, there are a number of other factors that can affect stress in animals. Some of these cannot be controlled directly, such as the weather, but their potential impact on stress in horses should be understood.

Environmental Effects: Extreme environments (high/low humidity or high/low temperature) has negative effect on health and welfare of the horse. The comfortable work environment for horses is estimated to be from 40°F to 77°F. A hot environment has a negative effect on both humans and horses by increasing sweating (leading to water loss) and respiratory rate, and decreasing feed intake. In winter, there is an increased risk of reduced water intake leading to colic in horses (Pritchard et al., 2006). All of these may increase stress in horses.

Other uncomfortable stimuli from environment, such as irritating color or noise, can cause stress in horses (Christensen et al., 2005). As a prey species, the equine sensory system is very sensitive to environmental changes. Equine high-frequency hearing extends far above that of humans but horses may be less able to localize the point of origin of brief sounds (Saslow, 2002). During one study, a novel sound (white noise, 10-20.000 Hz, 60 dBA) was played from a CD player which was hidden behind the horses feed container. Reactions to the noise test were compared to a suddenness test, where a fast moving white bag was used to stimulate horses. Results showed that exposure to a suddenly moving object caused more arousal behavior and higher heart rate responses. However, both stimuli can cause a fear response in horses (Christensen, 2005).

Transportation Related Factors: As prey animals, the horse is very sensitive to environmental changes. This includes horses moving to a new location. Both the transportation to the new location and the change in surrounds may result in fear and/or stress in the horse. Illness due to transport-related stress is a common problem in horses. One study evaluated the effects of long term-transport (24 hours) on stress indicators in horses. Increased cortisol concentrations and respiration rates indicate that horses experienced stress throughout the transport period (Guay et al., 2009). Another study measured cortisol concentrations and HRV in horses at the beginning, end, and one day after short transport (1 and 3.5 hours) and medium transport (8 hours). Results showed that both short and medium distance transport of horses led to increased cortisol release and changes in heart rate and HRV indicative of stress. The degree of these changes was related to the duration of transport (Schmidt et al., 2010). It was also found that transferring the young race horses from a familiar to unfamiliar environment caused the emotional responses which were shown by an increased HR during grooming (Janczarek and Kedzierski, 2011).

Feeding and Health Management: Nutritional supplementation depends on the needs of the horse. An inappropriate feeding, whether from unbalanced nutrients or improper feeding methods, can be reflected in changes in physiology and behavior. Some abnormal behaviors may serve as a coping mechanism, functioning to reduce stress or to provide the animal with some form of control over its environment (Mason, 1991; Cooper and Albentosa, 2005; Wickens and Heleski, 2010). Regardless the intent, these abnormal behaviors indicate stress. Crib-biting, also called cribbing or wind-sucking, is one of the most prevalent stereotypic behaviors in horses (McGreevy et al., 1995). Surveys have shown that low forage diets or high-starch/high concentrate diets can increase the risk of crib-biting (McGreevy et al., 1995; Wickens and Heleski, 2010). Wood chewing often happened with horses fed a diet higher in concentrates than in horses fed hay (Willard et al., 1977). Besides, crib-biting has been associated with excessive tooth wear and potentially life threatening colic (McGreevy et al., 1995). Horses in race training are commonly fed high-concentrate and low-roughage diets, and have a high prevalence behavioral stereotypies and gastric ulcers (Hammond et al., 1986).

Stress and Disease： Stress has a positive correlation with disease. Humans and animals experiencing stress can become more susceptible to disease, and being ill can cause stress in and of itself. Some diseases have a direct effect on hormone concentrations. Plasma cortisol concentration was lower in five Thoroughbred geldings after induction of a chronic inflammatory response, as compared to control horses (Mills et al., 1997). On the other hand, protracted laminitis often results in prolonged, elevated cortisol secretion (Johnson et al., 2004). In addition, horse with one diseases may be more stress sensitive. Management evoking stress in horses with gastric ulcers resulted in worsening symptoms (Malmkvist et al., 2012). Other research has shown evidence linking stress to chronic health problems, including reproductive complications, gastric ulcers, and weight loss (Budzyńska, 2014).

Effects of Stress

Stress is a physiological mechanism that allows a horse to adapt to threats, and response can range from superficial discomfort to death. Not all stress is bad, however. For example, glucocorticoids (one form of stress indicator) are released in response to situations that are not normally regarded as stressful, including courtship, copulation and hunting. The secretion of cortisol and epinephrine are linked to the fight-or-flight response, allowing animals to physiologically prepare to fight for their lives or flee as circumstances dictate. However, as mentioned earlier, long term stress can lead to suppressed immune systems (Minton, 1994; Dhabhar, 2000) and behavioral changes in horses. In particular, uncomfortable feelings such as pain, fear and anxiety are linked to overstress.

Behavior changes due to stress caused by pain is extremely common in horses (Dalla Costa et al., 2014). Pains which may come from muscles, joints, trauma, or internal organs are all possible sources of stress. Different types of pain in horses can result in abnormal behaviors. For instance, a sharp pain of sudden onset usually induces a reflex escape or attack reaction; head pain elicits head shaking, snorting, and restlessness; and back pain may lead to resentment of the saddle or grooming, sinking when the rider mounts, or failure to bend or yield to riding aids (Taylor et al., 2002). However, some smaller behavior changes are often overlooked or misunderstood. Many ridden horses are described as “lazy”, but it may be that some of these ‘‘lazy’’ horses are actually subject to low-grade pain (Hall et al., 2013). In addition, stress caused by chronic discomfort or pain may often be overlooked when facing “bad tempered” individual horses (Fureix et al., 2010). Also, invisible damage can be linked to stress. For example, the harm caused by internal parasites has been associated with long-term activation of the sympathetic nervous system as well as chronically elevated or depressed adrenocortical functions (Keeling and Jensen, 2002).

If horses are subjected to stress-related factors long term, then poor performance or loss of appetite (include picky eating or feeding habit change) can occur. For instance, race horse performance decreased due to horses not coping with their stress quickly enough (Alexander and Irvine, 2001). Stress can also have an effect on the equine reproductive system, decreasing libido by suppressing the sex hormones (estrogen in the mare or testosterone in the stallion) (Mills and Nankervis, 1999).

Stress Management and Welfare in Horse

Stress management is an important aspect of equine health care. Various methods, including pain reduction, increased turnout time, and allowing association with other horses, have been used to reduce stress in horses (FAWC, 1992). The Five Freedoms of animal welfare were initially established in the 1970's and has been modified several times. They include freedom from hunger, thirst, and malnutrition; freedom from discomfort; freedom from physical pain, illness, and injury; freedom to express the normal behaviors of the species; freedom from fear and anxiety are the general principles of equine welfare (FAWC, 1992). Addressing these "freedoms" may help improve equine welfare. However, because different horses are managed in different ways, subjecting them to different types of stress, no single management plan will work for all horses. Horse owners or managers need to pay attention to their horses' health and behavior in order to optimize equine welfare. Unfortunately, some of the assessment methods mentioned previously are either very costly or take time, possibly delaying implementation of better management strategies.

In an effort to determine stress levels in riding horses, two groups of animals were evaluated during routine use: one in a therapeutic riding program, and a second in a university riding program. Horses were evaluated for serum cortisol changes, and behavior was observed at several intervals during each program. It was hypothesized that changes in stress levels in horses would be detected by observing changes in serum cortisol concentrations and behaviors evaluated.

Materials and Methods

Experiment 1

Horses

Seventeen healthy adult horses, mean age of 14.7±10 years, participating in equine assisted activities and therapies (EAAT) at a therapeutic riding center (TRC) were used in this study. There were 15 geldings and 2 mares of mixed light horse breeding, the majority of which were Quarter Horse based. All horses had been owned by the TRC for at least three years prior to start of the study and regularly participated in riding activities. Horse management, including feeding, turnout, and routine health care, was not changed during the study. Also, no horses were removed from, nor did any new horses arrive at the facility during this time.

Riders

Although this study was deemed exempt from IRB oversight, legal consent was granted from riders or parents/legal guardians of all riders of horses involved. All riders at the TRC had some degree of physical, mental, or psychological disability. Standard practice at the TRC required each rider to have a handler for their horse for the entire lesson to ensure safety of the participant. Some horse-rider pairs also required one or two additional assistants, referred to as side-walkers, to provide additional support for the rider depending on the nature of the rider's disability. Nine different disabilities were observed during the duration of the study. Because rider disability could be a potential factor affecting equine stress evaluations, rider disability type was further classified in accordance with the Individuals with Disabilities Education Act (IDEA, 2006) into three subgroups in order to simplify statistical analysis. Group 1 consisted of participants with Attention Deficit Hyperactivity Disorder (ADHD), Epilepsy, and Other Health Impaired disabilities. Group 2 consisted of participants with Autism, Asperger Syndrome (Asperger's), and Sensory Processing Disorders. Finally, Group 3 included participants exhibiting Traumatic Brain Injury (TBI), Down Syndrome (Down's), and Cerebral Palsy (CP).

Riding Program

The riding program consisted of two consecutive 8 week riding sessions between January and May 2017, referred to as session A and B. Horses were allowed a 4-week recovery period before starting session A to account for winter time holidays during December 2016 and January 2017. Upon completing session A, horses were allowed a 2-wk rest period before starting session B. During each riding session, horses were used in 3 riding lessons per week. Each lesson lasted 1.5 hr for a total of approximately 5 contact hours per week. Each 1.5 hour riding session included approximately 45 min for tacking and untacking and 45 min for riding. Data was collected during weeks 1, 5, and 8 of session A and B. All riding lessons and data collections were held in a climatic controlled indoor riding arena. Horses were assessed and data collected during in accordance with standard operating procedures at the TRC during actual riding classes; no changes other than data collections were made to the lessons.

Behavior Evaluation

Equine behavior was assessed through both live audio and video observations using a modified behavior rubric (Kaiser et al., 2006; Denderen 2011; Collins et al., 2017) (Appendix B). Modifications to the rubric included reducing the complexity of the ethogram used by Denderen (2011) but including behaviors and descriptions as seen in Kaiser et al. (2006). The intent was to provide a rubric easily understood by both novice and advanced horsemen.

Behavior data was collected using a team of 4 individuals: one video recorder, one time/record keeper, and two behavior observers. The rubric used for behavior evaluations was tested in a pilot study (Collins et al., 2017) prior to its use in this project, and the two individuals observing horse behavior were trained in the use of the rubric before starting data collections. Both evaluators were experience horseman with a combined 25 years' equine experience.

The order in which horse were selected for observation was randomly generated for each data collection period throughout the study. Behavior evaluations were conducted during three 2-min periods during each riding class. The behavior team stood in close proximity to the riding arena to easily observe and record the riding lesson, while being careful not to disrupt the riding class or distract either the participants or the horses. Video and live audio recording were performed on the same horses simultaneously. Hand-held voice recorders were used to capture live behaviors then later transcribed to the behavior rubric sheet (Appendix B).

Behavior assessment included evaluation of equine head position, mouth, tail, and ear gestures, and locomotor and miscellaneous behaviors (Appendix B). The frequency in which horses repeated a behavior was recorded but not included in the statistical analysis for this thesis. Repeating a behavior for more than 5 seconds or more than 5 times during the 2-minute observation period resulted in the behavior being recorded as "continuous". Upon completing the 3 two-minute riding observations, observers gave a subjective overall behavior score ranging from 1 to 4 as follows: 1 = horses exhibited very few abnormal or negative behaviors during evaluation; 2 = horses exhibited few to a moderate number of abnormal or negative behaviors during evaluation; 3 = horses exhibited a moderate to high number of abnormal or negative behaviors during evaluation; and 4 = horses exhibited a high number of abnormal or negative behaviors during evaluation.

Blood Collection

Jugular blood samples were collected to determine equine stress based on changes in serum cortisol concentrations. Blood samples were collected using 20-guage needles and anticoagulant free 5 ml vacutainer tubes (Monoject™ blood collection tubes, Covidien LLC, Mansfield, MA, USA) before tacking (PRE), immediately following dismount of riders (POST), and 30 min post exercise (30-MIN) on alternate sides of the neck at each observation period during weeks 1, 5, and 8 of session A and B. Samples were allowed to clot at room temperature for 20 minutes before centrifugation at 2000 g for 10 minutes (ThermoFischer Scientific, Waltham, MA, USA). Serum was then pipetted into 1.7 mL microcentrifuge tubes (Argos Technologies, Vernon Hills, IL, USA), stored on ice, and transported to the Murray State University Breathitt Veterinary Center in Hopkinsville, KY. Determination of serum cortisol concentrations was conducrted within 24 hours of collection using a bench top immunoassay analyzer (IMMULITE 1000 Immunoassay System, Siemens Healthcare GmbH, Erlangen, Germany).

Statistics and Analysis

Statistical analysis was performed using SAS (SAS Inst. Inc., Cary, NC). Chi-square was used in evaluate differences between video and live audio recording and to evaluate consistency between observers. The Mixed procedure was used to determine the following: 1) differences in cortisol concentrations over time (pre, post, and 30 min), 2) effects of horse gender and age category on cortisol concentrations post and 30 min after riding, 3) effects of disability type of the rider on cortisol concentrations post and 30 min after riding, 4) changes in overall behavior scores throughout the study, and 5) effects of disability of the rider on changes in overall behavior scores. Significance was declared at $P \leq 0.05$, and trends at $P \leq 0.10$.

Experiment 2

Horses

Twenty-five healthy adult horses participating in in equitation classes at Murray State University (MSU) were used. There were 21 geldings and 4 mares of mixed light horse breeding, the majority of which were Quarter Horses. Age of horses ranged between 8 to 20 years, with a mean age of 14 years. Horses had been owned or used in the MSU equine program for 5 to 20 years.

Riders

Participants in experiment 2 consisted of Murray State students currently enrolled in equitation classes during the Spring semester of 2017. As standard operating procedure for all students, the skill level of the student was determined by the riding instructor prior to start of the semester to ensure safety of the riders. Riders were then classified according to their equine experience and riding abilities on a scale from 1 to 9, where a 1 indicated very little equine experience and a 9 indicating advanced experience. Rider scores were further evaluated retrospectively and simplified into three groups with riders in group 1 being inexperienced riders (Beginners, n=6), group 2 being intermediate riders (Intermediate, n=2), and group 3 being advanced riders (Advanced, n=2).

Riding Program

This study was conducted during a regular 16-week academic semester during the Spring 2017 from January through May. Data was collected during weeks 1, 9, and 15 with horses being used in 1 to 5 riding classes per week and each class lasting approximately 1.5 hr. Roughly 30 min of the allotted time involved tacking and untacking the horse while the remaining 60 min involved riding.

Behavior Evaluations and Blood Collections

The methods used for behavior evaluations and blood collections are similar to those described in experiment 1 with the exception that behavioral observations were made during tacking (one 1-min period), while riding (three 2-min periods), and then again while untacking (one 30-sec period). Overall scores (ranging from 1 to 4) and live audio and video observations were made as described in Experiment 1. Blood samples and serum cortisol concentrations are the same as those described previously with collections occurring during week 1, 9, and 15 of the study.

Statistics and Analysis

Statistical analysis was performed as described in Experiment 1, except that experience level of the rider was evaluated rather than disability type.

Results and Discussion

Results reported here are part of a larger study evaluating stress in riding horses. Overall, no horses showed signs of significant stress at any point during data collection.

Experiment 1: TRC

Changes in Serum Cortisol

Cortisol concentrations changed over time (Table 1), but values were within or slightly below normal ranges. The highest cortisol concentrations were observed in PRE samples (2.0425 µg/dL) and differed from those observed POST and 30 MIN after the ride (1.7860 and 1.4830 µg/dL, respectively; $P \leq 0.02$). The lowest cortisol concentrations were observed at 30 MIN and differed from POST values (1.4830 µg/dL and 1.7860 µg/dL, respectively; $P = 0.0107$).

Stress is associated with higher cortisol concentrations (Peeters et al., 2011). Normal serum cortisol concentrations for horses based on reference ranges for the laboratory used are 3 to 10 µg/dL (P. Godwin, personal communication, October 23, 2017). Serum cortisol concentrations remain stable with little change occurring when stored at 4ºC or room temperature (22- 24°C) for 72 h (Reimers et al., 1983). The fact that serum cortisol was higher before riding rather than after suggests that horses were not stressed by riding, but were perhaps anticipating the activity to come. Additionally, given that cortisol concentrations remained within or slightly below normal ranges and declined during in TRC horses suggests that horses were not negatively stressed due the riding experience.

Table 1. Serum cortisol concentrations in horses used in an equine therapeutic riding program.

Location	Serum Cortisol Concentration by Treatment (µg/dL)			
	PRE	*POST*	*30MIN*	*P-Value*
CCTRA	2.0625[a]	1.7860[b]	1.4830[c]	P<0.0001

Data presented as least squares means.
Different letters within the same row differ statistically.

Changes in Overall Behavior Scores

An interaction tended to exist between session and week for overall behavior scores (P=0.0664). Overall behavior scores were assigned by observers following each riding observation with a score of 1 indicating few negative or abnormal behaviors and a score of 4 indicating that a high degree of negative or abnormal behaviors were observed. Overall behaviors scores were highest during session B week 1, compared to session A week 8, session B week 5 and session B week 8 (1.4286, 1.1964, 1.1875, 1.1538 respectively; P≤0.01). There was a four wk break before starting session A, and a two wk break between session A and session B. These breaks were in place to account for holidays as well as to give the horses a mental and physical rest allowing for relaxation and recovery. Results showed that horses tended to express more negative behaviors when they returned to the program after the two wk break. Given the lower overall behavior scores in session B week 1 as compared to session A week 1 (P=0.0745), it is possible that a 2 wk break may not have been long enough for horses to recover. Since lower overall behavior scores were seen after a 4 wk break, a longer break between may be appropriate. That being said, overall behavior scores for session B week 5 and session B week 8 were numerically the lowest observed in the study. This suggests that horses adapted quickly to the resumption of the program. However, conversation with handlers as both session A and session B progressed suggested that horses were less well-behaved during tacking and thus may be a point of consideration for future studies.

Overall behavior scores in session A were similar, while overall behavior scores in session B differed ($P \leq 0.01$). There were no significant differences between overall scores for session A and session B. This result suggests that overall behavior score was not affected by riding session.

Although overall behavior scores differed statistically between weeks at certain points during the study, overall behavior scores were considered to be low with horses exhibiting little to no adverse or negative behaviors (Figure 1). The range for overall behavior score was 1 to 4. The actual overall behavior scores recorded were all between 1 or 2 (1.15 to 1.43, respectively) with no 3 or 4 scores observed in this study. The actual behavioral impact of such a small difference is difficult to ascertain. The low overall score suggests that horses were showing few negative behaviors, which would be linked to less stress. Therefore, horses in this TRC did not appear to experience stress as measured by overall behavior score.

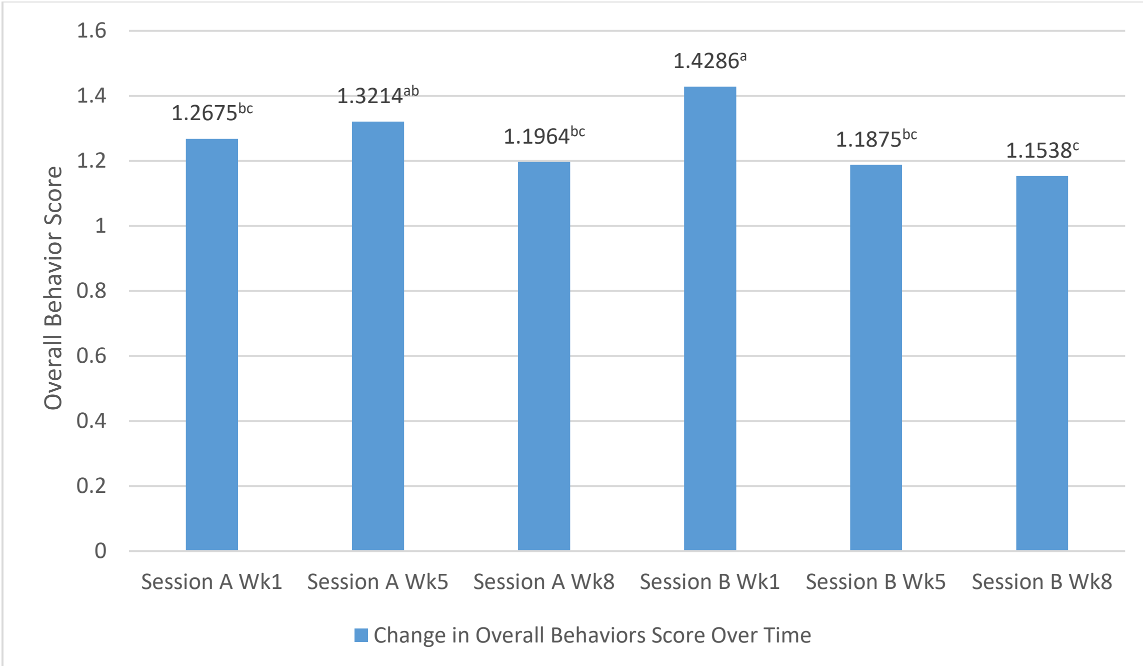

Figure 1. Overall behavior scores in horses used in a therapeutic riding program. Overall behavior scores with different superscripts differed, $P<0.05$.

Effects of Disability Type of Rider on Overall Behavior Scores

Disability type was classified into three categories in accordance with the Individuals with Disabilities Education Act (IDEA, 2006) in order to simplify the statistical analysis: Group 1: ADHD, Epilepsy, and Other Health Impaired disabilities; Group 2: Autism, Asperger's, and Sensory Processing Disorders; and Group 3: TBI, Down's, and CP. Results from this study suggested that equine behaviors may differ depending on disability type of their riders (Table 2).

Able-bodied riders can give horses conflicting signals, resulting in resistance and other negative behaviors in the animal. Individuals who have mental, physical, or psychological disabilities may not be able to control their physical movements easily, or may not be inclined to do so, and could therefore stimulate the horse in a negative way.

Although overall behavior scores remained low for the duration of the study, horses with riders belonging to Group 1 were noted to exhibit the highest behavior scores which were similar to Group 2 riders but differed from horses whose riders belonged to Group 3 (1.34, 1.29, and 1.17, respectively; $P \leq 0.05$). Riders in Group 3 included individuals who may have learning disabilities, intellectual disabilities, or movement disorders that limit their ability to move in ways that may confuse the horse. Riders included in Groups 1 and 2 included those who may have hyperactivity issues or delayed motor development leading to incoordination and clumsiness. Nevertheless, horses did not show high behavior scores during any session indicating that horses were not significantly stressed regardless of disability group. Thus, it is possible that horses with more active or enthusiastic riders may be more in tune or aware of the rider.

Table 2. Overall behavior scores in horses used in an equine therapeutic riding program.

Location	Overall Behavior Scores Per Group			
	Group One	*Group Two*	*Group Three*	*P-Value*
TRC	1.3437[a]	1.2875[a]	1.1700[b]	P=0.0504

Data presented as least squares means.
Different letters within the same row differ statistically.

Effects of Equine Age and Gender on Overall Behavior Scores

Overall behavior scores were not affect by age and gender of TRC horses (P=0.8876, P=0.8275, respectively). Most TRC horses were middle-aged or older (mean age 14.7 years). Younger horses are commonly more reactive and unpredictable, so it would be appropriate to evaluate younger horses before assuming the results of this study.

Experiment 2

Changes in Serum Cortisol Over Time

Serum cortisol concentration were highest upon removing horses from their stalls or pastures for tracking and were similar to POST cortisol concentrations observed immediately upon dismount of riders. (2.9606 and 2.6880 µg/dL; P=0.18930). Within 30 mins following dismount of riders, cortisol concentrations declined and differed from PRE and POST concentrations (2.1314 µg/dL; P<0.0079) (Table 3).

Normal serum cortisol concentrations for horses based on reference ranges for the laboratory used are 3 to 10 µg/dL (P. Godwin, personal communication, October 23, 2017). Values in this study were often higher than 2 µg/dL and lower than 3 µg/dL. The fact that serum cortisol was higher at PRE and POST as compared to 30 MIN (P=0.0004) suggests that horses experienced some degree of stimulus to being caught and during riding. However, all serum cortisol concentrations were within a normal range, so horses in the riding class did not appear to be experiencing extreme stress as measured by serum cortisol concentrations. The higher PRE cortisol concentration may have been caused by horses anticipating the exercise, which could be defined as a "good" stress response.

Table 3. Serum cortisol concentrations in horses used in the MSU riding program.

Location	Serum Cortisol Concentration Per Treatment (µg/dL)			
	PRE	*POST*	*30MIN*	*P-Value*
MSU	2.9606^{a}	2.6880^{a}	2.1314^{b}	P=0.0004

Data presented as least squares means.
Different letters within the same row differ statistically.

Cortisol concentration was highest during week 9 and week 15 compared to week 1 (2.9709 µg/dL, 2.9054 µg/dL, and 1.9039 µg/dL; $P \leq 0.0001$) (Table 4). Suggesting that horses were less stressed at the start of the semester. This coincides with the activity level of the riding class where increased demand is placed on the horses as the semester progresses. Horses used in college program were only asked to do very simple movements during week 1, but more intense exercise was required as the course progressed. When reviewing the video recordings, horses were only asked to walk in week 1, but were asked to walk, trot, and canter during weeks 9 and 15. Therefore, the difference in cortisol concentrations between week 1 and weeks 9 and 15 ($P<0.001$) may have been caused by the increased amount of exercise and would be considered a normal increase and not a response to negative stress.

Table 4. Serum cortisol concentrations in horses used in the MSU riding program.

Location	Serum Cortisol Concentration Per Study Week (µg/dL)			
	Week 1	*Week 9*	*Week 15*	*P-Value*
MSU	1.9039[a]	2.9709[b]	2.9054[b]	$P<0.0001$

Data presented as least squares means.
Different letters within the same row differ statistically.

Changes in Overall Behavior Scores Throughout the Semester

There were no significant differences in behavior scores over time ($P=0.1080$). Overall behavior scores were similar throughout the study, which suggests that negative behaviors were not expressed more frequently as the riding class progressed. Indicating that horses were not stressed during the riding class. This result was contrary to the changes in cortisol concentration, which showed an increase from week 1 to weeks 9 and 15. Cortisol concentrations have been shown to increase with exercise (Kędzierski et al., 2013), which could be considered as a form of “positive stress”. It is simply reflecting the natural hormonal response. With that in mind, few negative behaviors were observed resulting in no change in overall behavior scores. It is possible that small changes in stress levels are more detectable by evaluating serum cortisol than overall behavior scores. It may be useful to repeat the study using horses that are in a more demanding exercise program in order to potentially generate more negative behaviors. A larger scale for the overall behavior score, perhaps 1 to 10 instead of 1 to 4, may reveal subtler changes in observed behaviors. Finally, it would be appropriate to use more observers with more equal experience levels before comparing more and less experienced observers.

Effects of Equine Gender and Age on Overall Behavior Scores

There were no differences observed in overall behavior scores for younger versus older horses (1.2209 and 1.1633; P=0.4096) but gender tended to affect overall behavior scores in MSU horses, with mares showing more negative behaviors (P=0.0892). This result may have been caused by one particular horse who demonstrated continuously displayed negative behaviors, such as ears being pinned back, during each ride. According to faculty and management staff, that is the normal behavior for this mare and not an adverse response due to design or implementation of the study. However, due to the small sample size, data points from this individual were not able to be excluded from the analysis.

Effects of Experience Level of Rider on Overall Behavior Scores

Despite the fact that more than half of the riders in the class were classified as beginners (n=6/10), there were no differences observed in overall behavior scores based on experience level of the riders. Horses used in college riding class were all well trained horses, and most of them had been owned by the university for more than 5 years. Therefore, the horses involved in this study were familiar with the riding class. Suggesting that the length of time the horse has been involved in a riding program may be more appropriate for consideration in future studies. In addition, skill level of the rider did not affect overall behavior score.

Effects of Experience Level of Rider on PRE/POST/30MIN Cortisol Concentration:

Experience level of the rider had no effect on PRE, POST, and 30MIN cortisol concentrations which is in agreement with the previous results from overall behavior score. Further studies may be needed to determine if rider experience level negatively impacts stress in horses.

Audio versus Video Observation

Comparison of audio and video observation were made to determine accuracy of live observations and to ensure accuracy between observers. Live audio recording are made by two trained observers based on observation of behaviors during a two minute riding period unlike video observations where the observer has the ability to pause or play back footage ensuring no behaviors are missed. No differences between observers for audio recording ($P=0.8234$) or video recording ($P=0.6069$). Furthermore, overall behavior scores were similar between audio and video recording ($P=0.8767$). This high degree of similarity between live and video observation is encouraging because it allows for rapid determination of behavior and stress in riding horses. Thus, the audio or video option may be used independently in the future studies, but, it should be noted that only two observers were evaluated in this study.

Results also suggest that level of horse experience did not have an effect on assignment of overall behavior scores. While this applies to overall behavior scores, it may not apply to more specific behaviors noted. For example, two observers may give the same overall score, but the observed behaviors may be explained differently. One observer may note “tail raised”, while the other may note “tail swish”. Depending on the circumstances, one may be a neutral behavior (“tail raised”) while another could be attributed to a negative behavior (“tail swish”). The observer with less experience tended to give a more detailed explanation of observed horse behaviors. For example, whereas the more experienced observer may have noted 3 “ear forward” behaviors, the less experienced observer may have detailed 2 “ear play” and 1 “ear forward”. The total number of ear behaviors noted was the same, but the actual behaviors detailed may have been different.

Comparison of Experiments 1 and 2

The TRC and college riding class evaluated in this study both showed that horses had lower cortisol concentrations, which is associated with less stress. Additionally, overall behavior scores were reported between 1 or 2 during both experiments. This was linked to a low incidence of negative behaviors and, therefore, less stress in horses. The two different methods used to measure stress had similar results – that is, horses in these programs did not seem to be experiencing significant stress. Data suggests a possible relationship between serum cortisol concentration and horse behavior changes, with considerations mentioned above.

Both experiments showed a higher cortisol concentration before riding began and lower concentrations at 30MIN. The higher cortisol concentrations were associated with removing horses from their stalls or pastures and bringing them into the tacking area. This suggests that horses may have been anticipating the activity to come but then relaxed as a familiar event (riding) occurred.

Rider experience level had no effect on overall behavior scores or cortisol concentration. However, rider disability type did have an effect. Beginning riders are often unbalanced while on horseback and are untrained in giving correct cues to the horse. Since not all the riders in the university course were beginner riders, it is possible that the ability of the more advanced riders masked the effect beginner riders may have had on the horses. Disabled riders may also be unbalanced and, depending on the disability type, may be unable to give clear cues to the horse. It is possible that disabled riders may be more unbalanced, or become unbalanced more frequently, and give frequent miscues compared beginning riders resulting in increased stress on the horse. Further studies are needed to clarify how rider disability can impact equine health, stress, and behavior.

Both experiments also indicated that horse gender and age did not affect overall behavior score. However, the horses involved in these two studies were all middle-aged or older horses. Whether at the university or TRC, only horses with a gentle temperament that were well-trained were allowed to participate in beginning or therapeutic riding programs. Most of the horses in this study had been involved in the programs for more than 3 years, so they were familiar with the routine. These horses were not only well trained, but also had adapted to the riding class. Numerous studies show that equines adapt to stress and the more experienced they are, the less stressed they are (Budzyńska, 2014; Cooper, 2005).

Conclusion/Implications

Horses used in a university beginning riding class or at a therapeutic riding center did not show signs of negative stress as evaluated by serum cortisol concentration or overall behavior scores. However, analysis indicated the possibility of stress from changes in the level of exercise in university riding horses. No effects were found based on rider's skill level, but different disability types affected horse's stress at the TRC. This indicates that certain types of rider behavior may have a negative effect on horse's behavior, but further investigation is needed. A relationship between stress-related behavior and cortisol concentration changes was not shown clearly in this study. However, neither of the riding programs evaluated demanded a lot from the animals, placing them in a low-stress environment. That being said, horses working harder or longer, or those in a higher-stress environment, may show a clearer relationship between behavior and cortisol concentrations. Further studies need to be conducted to better understand the impact of riding on equine stress.

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Appendix A: Table of horse stress reactions (Kaiser et al., 2006)

Ethogram of behaviors observed in horses while being ridden in a therapeutic riding program.

Behavior	Description
Head toss*	Head lowered with the ears pinned back interrupted with momentary sharp tossing or rotating gestures of the head.
Ears pinned back*	Ears pressed caudally against the head and neck.
Head raised*	Head held higher than the normal carriage with nose extended upward and with slight extension of the neck.
Head down*	Head held lower than the normal carriage; neck may be stretched out with nose pushed forward.
Ears turned (listening)	Ear movement from pointing forward to pointing backward; may be unilateral or bilateral.
Head shake*	Repeated rhythmic, mild flipping motions of the head.
Head turn*	Moving head left or right independent of the rider.
Moving tail	Any exaggerated movement of the tail, usually more of a wringing motion than a rhythmic or directed swishing.
Chomping bit	Any mouth or tongue manipulation of the bit independent of the rider's use of the reins.
Whinny (neigh)	Loud, prolonged (typically 1 to 3 seconds) call beginning high pitched and ending lower pitched; head is elevated and the mouth opened slightly.
Moving backwards	Backwards movement of the horse in a 2-beat gait with diagonal pairs of legs working together (trotting in reverse).
Halt	Cessation of movement of all 4 feet.
Walk	An even 4-beat gait in which the sequence of beats is lateral in that both feet on 1 side strike the ground before the feet on the opposite side strike the ground.
Trot	A 2-beat gait in which diagonal pairs of legs strike and leave the ground simultaneously.
Canter	A 3-beat gait in which the first and third beats are made by 2 legs striking the ground independently and the second beat is made by 2 limbs striking the ground simultaneously.
Defecation*	Expelling of feces; the anal sphincter contracts rhythmically, and the tail is raised and may be lashed vertically at the completion of defecation.
Urination (male)	With forelimbs slightly extended forward and hind limbs extended backward and slightly spread, expelling of urine.
Urination (female)	With the back arched, the tail raised, and the hind limbs extended backward and slightly spread, expelling of urine.

*Behaviors classified as stress-related behaviors. More extreme behaviors indicative of stress while being ridden, such as bucking or rearing, were never observed, and none of the horses ever attempted to bite, strike at, or kick a rider, a leader, or a side walker. Movement of the tail was not classified as a stress-related behavior because of difficulties in differentiating tail swishing as an irritation behavior from tail swishing as a response to flies. Chomping the bit was not classified as a stress-related behavior because not all horses wore bridles with bits during therapeutic riding sessions.

Appendix B: Modified Behavior Rubric (Collins et al., 2017)

Horse ID/Name: ______________ Date: ______________

Class Type: ______________ Location: TRC MSU

Observation: Audio Video

In the columns below, enter the total number of times a given observation is noted in each audio or video recording

Behavior Rubric Checklist

Head Position

Ride Obs	**1**	**2**	**3**
Neutral			
Flexed			
Pulling			
Low			
Ground			
High			
Tilted			
Turned			
Toss			
Shake			
Stretch			
Not visible			

Mouth Gestures

Ride Obs	**1**	**2**	**3**
Neutral jaws			
Tense jaws			
Play w bit			
Gaping jaw			
Tongue lolling			
Tongue out			
Not visible			

Ear Gestures

Ride Obs	**1**	**2**	**3**
Neutral			
Earplay			
Forward			
Backward			
Pinned			
Not visible			

Tail Gestures

Ride Obs	**1**	**2**	**3**
Neutral			
Swishing			
Tense			
High			
Not visible			

Overall Impression score: ______

1 - 4 with 1 being relaxed, 4 being tense

Locomotor behavior

Note when changes of gait are requested as opposed to being initiated by the horse

Ride Obs	**1**	**2**	**3**
Neutral velocity			
Faster velocity			
Slower velocity			
Halt			
Backwards			
Fast backwards			
Abrupt halt - reluctant to move			
Change in gait			
Crabbing			
Counter canter (crossfiring)			
Changes leads			
Helper encourages			
Helper slows			
Backlash (bucking, rearing, etc.)			
Stumbling			
Falling			
Turning			
Shying			
Defecation			
Blowing			
Snorting			
Groaning			
Sighing			
Coughing			
Not visible			

Miscellaneous

Ride Obs	**1**	**2**	**3**
Strider (noise in airway)			
Showing the whites of the eyes			
Nose/eye not visible (No visible/audible signs seen or heard)			

Notes: