EXPLORING HORSE REACTIVITY AND HABITUATION ACROSS WORK TYPES

Chloe C. Wires

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Horse Science Equine Education

Middle Tennessee State University

May 2017

Thesis Committee:

Dr. Rhonda M. Hoffman, Chair

Anne M. Brzezicki

Dr. John C. Haffner

ACKNOWLEDGEMENTS

I would like to thank Dr. Rhonda Hoffman for her encouragement and support throughout my graduate career. The knowledge and experience I have gained from her is invaluable. She continues to rise above and beyond her responsibilities as an educator and her devotion to her students is truly admirable.

I would like to thank my committee members, Anne Brzezicki and Dr. John Haffner, for their support and guidance. Additionally, I would like to thank my fellow graduate students for helping me make this study a success.

I would like to thank Officer Jon Levi and the rest of the staff from Rutherford County Mounted Patrol for allowing me to use their horses in my study.

Finally, I would like to thank my mother and father for their love and support.

ABSTRACT

Previous research indicates horse behavior is affected by level of training, within the same type of work. The purpose of this research was to identify patterns of behavior in horses trained for different types of work. Eighteen adult horses trained for mounted patrol, equine-assisted activities and therapies (EAAT), or show, were evenly selected into three groups respective to type of work. All horses were individually turned loose in an 18 m diameter round pen and exposed to one of three novel stimuli (fog machine, 5.2 m tall air dancer, paintball gun) having visual and auditory components. For reactivity assessment, each horse was fitted with a heart rate (HR) monitor and Fitbit. Each horse was given a 30 s adjustment period to the round pen. Following the adjustment period, baseline reactivity was measured over an additional 30 s. Exposure to each stimulus was temporarily ceased for 60 s. Exposure to each stimulus resumed and habituation was measured. Heart rate was recorded every 10 s and the Fitbit step count was recorded immediately prior to entering and after exiting the round pen. A mixed model with repeated measures (SAS) was used to analyze effects of work type on heart rate, habituation, and steps. No difference in HR was reported when exposed to the air dancer ($P > 0.45$). Patrol HR was greater than EAAT ($P = 0.023$) and Show ($P = 0.012$) with no difference between EAAT and Show ($P = 0.77$) when exposed to fog. Patrol HR was greater than EAAT ($P = 0.046$) and Show ($P = 0.027$) with no difference between EAAT and Show ($P = 0.79$) when exposed to the gun. No difference in habituation was reported between work types when exposed to fog ($P > 0.43$) or gun ($P > 0.82$). EAAT habituation tended to be greater than Show ($P = 0.073$) when exposed to the air dancer. No difference in steps was reported when exposed to the air dancer ($P > 0.76$) or gun ($P > 0.77$). Patrol steps were greater than EAAT ($P = 0.017$) and Show ($P = 0.014$) with no difference between EAAT and Show ($P = 0.93$) when exposed to fog. These results agree with previous studies while also showing that type of work affects reactivity when exposed to novel stimuli. This study documented reactivity of horses that may be applicable when selecting horses for certain jobs.

Key words: Behavior, Stimuli, Habituation

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CHAPTER I: LITERATURE REVIEW

Introduction

For centuries, the relationship humankind shares with the horse have gone above and beyond that of a mode of transportation, workmate, or creature of livestock. To many individuals, the horse has become a true companion. Horses represent power, courage, and unbridled passions (Frewin and Gardiner, 2005). They are considered as heroes and warriors to many. Humankind is drawn to their irresistible beauty and strength.

Jones (1983) suggested that the horse represents "human spirit and freedom." Kohanov (2001) discusses the relationship of the horse with humankind as "a single herd of mutual influence and cooperation". Anyone who has had the remarkable opportunity to work individually with a horse can attest to the powerful bonding experience. The positive interactions between horses and humans can be quite beneficial.

Horses provide us with a different form of relationship that other animals cannot. They are prey animals, and therefore do not trust others easily. In order for horses to trust us, there must be a mutual level of respect. We must understand that we are putting them in an unnatural setting by ways of domestication. At the same time, they must understand that, with welfare in mind, we are not trying to hurt them in any way (McCormick and McCormick, 1997).

By mutual respect, a positive relationship is engaged by the horse and the human. Typically, horses are bigger and stronger than people. When working with these large creatures, there is a level of safety that is demanded, and with that people are more likely to retain attentiveness around these animals. Horses possess a natural ability to mirror our behaviors (McCormick, A. and McCormick, M., 1997) and there is a tremendous opportunity for learning more about these particular behaviors.

Natural Behavior in Horses

Horse behavior continues to intrigue horse-lovers, and has since ancient times (McGreevy, 2004). By nature horses are prey animals and their instinctive reaction is by means of fight or flight. Their survival is widely dependent of this instinctive behavior and when given the choice they will choose to flee. Horses will associate most experiences by either something safe to explore or something frightening to run from. Understanding this behavior provides us the possibility to possess a complete understanding of the horse. (Williams, 2008)

It is often overlooked that many of the requests we ask of the horse are in disagreement with their natural behavior. Horses have an impressive ability to adapt to these requests, allowing them to serve multiple roles in a variety of ways (Skipper, 2007). Hogg (2003) describes horses as simple creatures, but cautions they are easy to misunderstand. She makes the point that it is our responsibility to understand the horse, not the other way around.

The horse is one of many species that we ask a great deal of requests. Horses were once seen as having been set upon this planet purely to serve us. Horses are easily dominated due to them being herd animals where a dominance hierarchy is always established (Williams, 2008). Hogg (2003) brings to question, should the relationship be built around dominance or co-operation? Although domestication is not natural to the horse, their psychology has evolved allowing them to adapt to their environment. These changes are less advantageous to the horse and several practices today deprive them of their ability to act naturally (Skipper, 2007). Despite these changes, their need to act naturally still remains.

Stereotypic Behavior in Horses

A stereotypy is defined as any compulsive behavior that is expressed, despite not serving any ideal purpose or function (Nicol, 1999). Stereotypies can be categorized as oral, locomotive, or self-mutilating. The list of identified stereotypies is quite extensive, although some of the most common are cribbing or wind-sucking, pacing or weaving, stall walking, pawing, stomping, wall kicking, and self biting (Pascoe and Houpt, 2015). Although the exact cause of stereotypic behavior is unknown there are a variety of contributing factors, including management techniques, environment, and genetics. Although most stereotypic behaviors are not of concern to horse owners, they do provide insight into the behavioral mechanisms of the horse (McGreevy, 2004).

Clegg et al. (2007) evaluated seventeen adult Thoroughbred geldings, five of which were cribbers, six were weavers, and six were non-stereotypic controls. The cribbers and weavers had been identified stereotypic for at least one year prior to the study. After given a seven-day acclimation period, testing consisted of two sixteen-week periods. All horses were stalled for twenty-two hours with two hours of paddock time. Horses were fed at 10:00 and 17:00 hours. Results suggested that cribbing was most frequent during and after feedings and weaving was most frequent prior to feeding and turnout time.

Kusunose (2010) assessed the influence on feed type in relation to cribbing frequency. Three Thoroughbreds were evaluated and fed four meals per day: a pelleted concentrate and oat mix (3-4kg) at 06:30 and 16:00 hours and timothy hay (4 kg) at 09:30 and 19:00 hours. Cribbing frequency was greater post feeding of both roughage and concentrate, although the frequency was greater post concentrate. These results appear to agree to the study mentioned above by Clegg et al. (2007) in that cribbing is more frequent post feeding.

Key Terms of Behavior

When exploring behavior, regardless of species, it is important to have a clear understanding of key factors. Terms are often confused, which may elicit a different perspective on behaviors being expressed. While developing the horse-human relationship, the success of this relationship is important for the use of these animals in our daily lives.

Temperament

The manageability of a horse is highly dependent on their overall behavior and temperament. Calviello et al. (2016) defined temperament as individual differences in behavior that are present in early in life and relatively consistent through different situations. The performance and suitability of the horse in equestrian activities can be affected by the temperament of the horse. From a simplistic view, it is understood that gender, age, and environment also influence the temperament of a horse. Mills (2005) compared the temperament of stallions, geldings, and mares, and concluded that stallions were typically stronger both physically and mentally, whereas geldings and mares where considered more docile in comparison. She acknowledged that although it may be difficult to associate age with behavioral differences, there were still some generalizations that could be made. Younger horses were generally more playful and reactive compared to mature horses (Mills, 2005). It is assumed that horses receive more training as they get older, influencing their behavior.

Fear and Anxiety

A horse's response when placed in a fearful situation is of key importance to the safety of not only the horse, but surrounding handlers as well. Forkman et al. (2007) defined fear as a reaction to the perception of danger, whereas anxiety was defined as the reaction to a potential danger. There is a lack of good fear tests for horses that can accurately quantify these variables (Forkman et al., 2007). Horses manage fear in one of two ways, either choosing not to fear something, in which they will either ignore or explore it, or choosing the response of fear, in which they will enter flight mode (Williams, 2008).

Influences on Horse Behavior

Despite the ability for horses to quickly adapt to new environments, a horse may still face behavioral difficulties when introduced to new management practices. In addition to instinctive reactions, there are several other variables that have an influence on the behavior of the horse. Although there is limited research in these areas, the desire to explore horse behavior is continually growing. In order to fulfill the complete understanding of horse behavior, it is important to consider several factors.

Genetics

The genetic correlation to horse behavior is a relatively new research area, but is rising nonetheless. Two of the more common areas being explored today include phenotypic and stereotypic influence on the behavior of the horse. In the horse industry, bloodlines are commonly linked to particular jobs or equine disciplines, such as racing speed, cow sense, gait performance, conformation traits, jumping or dressage ability. The genetic makeup of a horse plays a vital role in the selection of these animals when being introduced to new management practices and training programs. This supports implementations for future research to accurately identify these genetic correlations to support better selection of horses used for specific work.

Finn et al. (2016) explored the relationship between coat color phenotype and horse behavior to determine if there were behavioral differences between chestnut horses and bay horses. An international survey was sent out to horse owners asking them to evaluate their own horses by answering a series of questions. In combination with these questions, each horse's behavior was assessed during handling, during exercise, and when separated from other horses. It was concluded that age, breed, and sex of the horse affects behavior, but no results proved any differences between bay and chestnut phenotypes. When comparing coat color phenotypes in relation to general behavior, chestnut horses were more likely to approach objects and animals regardless of their familiarity (Finn et al., 2016).

Continuing on with this phenotypic speculation, Brunberg et al. (2013) compared a population of Icelandic horses to determine if coat color influences behavior when exposed to novel stimuli. Horses with the Silver mutation Arg618Cys in PMEL show stronger fear reactions than horses without the mutation. The proportion of silver horses that were hesitant to entering the testing area was greater than the other phenotypes. Although the proportion of silver horses being hesitant to entering the testing area was greater, there were no differences in fear reactions to the stimulus or latencies to resume feeding. Although these results did not support Brunberg et al. (2013) hypothesis, the results agree with the previously mentioned study by Finn et al. (2015). Both studies displayed minor behavioral differences among various phenotypes, although there were no major behavioral differences when exposing these horses to novel stimuli or unfamiliar environmental factors. Level of training was not a factor in these studies, but insight into the general correlation between coat color and behavior is provided.

Hemmann et al. (2014) determined the heritability of crib-biting behavior. This study was the first to be conducted to determine the heritability of this specific stereotypic behavior. Crib-biting behavior expressed in the Finnhorse appeared to be similar to expressed behaviors in other breeds. As sire variance accounts for a quarter of additive genetic variance, Hemmann et al. (2014) estimated heritability of crib-biting was 0.68.

As this is the first study that estimated heritability of crib-biting behavior, there is a lot of speculation. Hemman et al. (2014) used a single breed and a small population. Therefore, no generalization could be made for all horse breeds. The high heritability of crib-biting in this study supports the assumption that a horse inherits behavioral susceptibility, predisposing it to stereotypical behavior phenotypes. Although this stereotypic behavior is not lethal to the horse, it is highly undesirable and is taken in to high account when breeding is considered. (Hemmann et al., 2014)

Nutrition

As an herbivore the horse is well suited for a high forage diet. As we continue to interfere with the natural feeding behavior of the horse by supplementing grain and fencing pastures, we are in turn affecting other behaviors as well. Just as in any other case these behaviors must be monitored and handled appropriately.

The supplementation of grain into the diet of the domestic horse is a common practice in feed management today. Many horse owners use this supplementation to account for the extra calories needed for performance demands. The horse can only tolerate so much starch in their diet (Harris, 2005). The consumption of large amounts of starch and sugars has been suggested to cause excitable energy in the horse (Greiwe et al., 1989). With the expression of this unnecessary energy, if not properly managed, not only can it cause stress to the horse but it may also lead to horse-related accidents or injuries.

Freire et al. (2009) studied the effects of two different amounts of grain and horses that were fed a high (50%) grain diet, compared to those fed a moderate (35%) grain diet, displayed an increase in the time it took for them to consume their ration, although there were no differences in reactive behaviors expressed. The diet had no effect on the frequency of stereotypic behavior when evaluating cribbers and weavers.

Macleay et al. (1999) compared behavioral responses of horses fed a high-carbohydrate or high-fat diet. Horses fed a high-carbohydrate diet were more reactive in their stalls, more difficult to catch and lead, and had higher heart rates. The proportion of fat in the diet can have an effect on heart rate and behavioral responses related to stress and startle reactions (Redondo et al., 2009). Horses on the fat diet had less intense startle reactions and their heart rates showed less increase when startled.

Bulmer et al. (2015) evaluated the responses of horses to novel stimuli and handling tests when fed a high-starch or high-fiber diet. For the novel stimuli testing there was no effect of diet on novel movement or heart rate reactivity. For the handling testing there was no effect of diet on handling movement or handling co-operation, however there was an effect on heart rate reactivity, as the horses on the high-starch diet expressed an increase in heart rate reactivity compared to those on the high-fiber diet.

Effects of Work and Training on Horse Behavior

Horses continue to serve a large variety of roles in our world today. As we continue to further the horse-human relationship, we must understand how certain management practices affect the behavior and welfare of these horses.

Mounted Patrol

Horses have become a strong attribute to the police force due to their great strength and size. The temperament of these horses is important to their level of reactivity under stressful situations as they are often used for crowd control as well as search and rescue activities. (Munsters et al., 2012). Police horses must be able to remain calm in stressful situations, as the level of needed safety extends beyond just the horse (Thomas, 2010).

The assessment of police work is crucial to avoid behavioral problems and improve welfare of horses used in this line or work. Munsters et al. (2013) assessed workload and stress of police horses during night patrols and riot control training, using heart rate, speed, plasma lactate concentration, and behavioral scores. The influence of the rider on fear response was explored as well as whether heart rate alone is an accurate indicator of stress during transportation. As the workload of these horses was low during training scenarios, low amounts of stress were expressed during riot control training and night patrols. Because of this, the use of behavioral scores was deemed useful only if the situation was challenging enough to provoke higher reactivity. During testing trials, horses elicited higher heart rates when not being ridden compared to horses that were being ridden. It was concluded that the interaction between the horse and rider did play a role in the stress levels elicited by the horses when presented challenging situations, as these horses were more reactive when they did not have a rider controlling them. (Munsters et al., 2013)

Aside from the workload expected from horses used in mounted patrol, undesirable behaviors and stress may be exhibited from other management practices. Pessoa et al. (2016) evaluated the effect of daily stall confinement time on the behavior and welfare of mounted patrol horses, proposing that confinement would increase the occurrences of undesirable behaviors, therefore increasing accidents during mounted patrol sessions. Horses were subjected to either total or partial confinement, and behavior was evaluated by direct observations and a questionnaire by military personnel during patrols. The horses under total confinement expressed high heart rates, in addition to higher cortisol values, compared to horses under partial confinement. Confinement type did have an effect on certain behaviors expressed as well. It was therefore concluded that turnout is a simple and effective practice that promotes horse welfare and positively influences behavior during police work.

Equine Assisted Activities and Therapies

The majority of the literature on equine assisted activities and therapies (EAAT) is primarily based upon case studies, survey responses, or theoretical literature (Ratliffe and Sanekane, 2009). Therapeutic riding was promoted for the reduction of physical disabilities, the improvement of psychological wellness, and the rehabilitation of people with below average motor control, and had been standardized by the Europeans (Tyler, 1994). The effects of therapeutic riding have also been directed towards issues including mood disorders, attention deficit disorders, communication problems, behavioral issues, substance abuse, eating disorders, abuse concerns, and psychosocial disorders (Shultz, 2005).

Recognizing that the use of EAAT positively benefits humans, it is important that we keep the welfare of horses used in this line of work a top priority. Anderson et al. (1999) evaluated the temperament and reactivity of therapy horses by comparing objective opinions of riding instructors in addition to cortisol, norepinephrine and epinephrine levels. Riding instructors traveled to four different therapeutic centers and the agreement between the instructors regarding the temperament of the horses evaluated was relatively low, having an average of 33%. Thus, therapeutic riding instructors should collaborate more when assessing the temperament of horses used in their programs. Reactivity testing data showed that 64% of the horses with the highest reactivity scores were used as therapy horses. Therapeutic riding centers need to be cautious about the common misconception that all therapeutic riding horses are well desensitized to stimuli. There were no correlations between cortisol, norepinephrine and epinephrine concentrations and temperament or reactivity of horses, although some trends indicated that temperament may be assessed on extreme values of reactivity or hormone levels.

Although it is commonly perceived that horses selected for therapeutic riding programs are more desensitized to stimuli, there is limited research that objectively measured reactivity in these horses. Minero et al. (2006) assessed the reactivity of therapeutic riding horses in comparison to jumping horses, exposing them to two challenges while comparing behavioral and physiological responses. For the first challenge, horses were tied in their stall and their heads were covered with a hood. For the second challenge, horses were loose in their stall and exposed to a novel stimulus (red and white garland on the end of a pole). No differences between cortisol levels of the two groups were reported. Despite there being no differences between the behaviors expressed and heart rate variability, the average heart rates of the therapeutic riding horses were greater than the jumping horses. These findings support Anderson et al. (1999) in that it can be concluded that horses used for therapeutic riding are desensitized to specific stimuli involved in their daily work, but are still reactive to novel stimuli.

The assessment of the autonomic nervous system and its regulation of cardiovascular function may be used as an indicator of stress in horses (Von Borel et al., 2007). Using this knowledge, Gehrke et al. (2011) evaluated heart rate variability of various frequencies in horses involved in EAAT to determine if heart rate variability is a valid measure to evaluate the suitability of a given horse-human pair involved in EAAT. All horses were observed over a 24-hour period with access to turnout. A decrease in average heart rate during nighttime compared to daily activities was reported. This was expected, as horses are typically not as active during nighttime hours. Results also indicated that heart rate variability oscillations in horses engaged in EAAT are within the same frequency ranges as human beings. These results support that direct comparisons can be made between human and horse HRV patterns, providing a quantitative method of determining the responses between the two species (Gehrke et al., 2011). This knowledge could be further used to determine if the emotional state of the horse can be influenced by the interaction between the horse and human.

<u>*Show or Competition*</u>

The horse-human relationship plays a vital role in both competition as well as recreational riding, yet there is a limited amount of scientific data to support the importance of this relationship. Despite the ongoing journey to understand the horse-human relationship, horse-related injuries do occur among professional and non-professional horsemen (Hausberger, 2008). Keeling et al. (1999) determined the relationship between the horse and rider can influence the risk of injury while riding, as approximately one quarter of all horse-related accidents was due to fright responses by the horse and miscommunication between the horse and rider. Therefore, identifying factors that influence behavioral responses by the horse may reduce the number of horse-related accidents.

Borstel et al. (2010) evaluated differences in fear reactions with regard to reaction strength and habituation between trained and untrained horses engaged in dressage and show-jumping. Reactivity responses were classified in five categories: Flight, Sidesteps, Alert, Head up, or None. Results shown that habituation did occur, as there was a decline of reactive responses among the five testing trials, although horses that were ridden more than half a year (deemed "trained") habituated faster than "untrained" horses. Habitation was not correlated to genetic lines of dressage or show-jumping. Horses that were more reactive to one trial were more reactive in other trials. Analyzing the data from the stimuli testing, results from this study supported the impression that dressage horses are more easily frightened than show-jumping horses (Borstel et al., 2010). These results agree with Hausberger et al. (2004) findings that dressage horses elicited higher levels of anxiety when compared to show-jumping horses. This is indicative of low numbers of accidents with show-jumping horses as they typically respond less in fearful situations.

The importance of behavior traits such as temperament is driving research to move from subjective scoring towards a more objective way of analyzing reactive behaviors in horses. Borstel et al. (2011) compared heart rates and behavioral responses in the same temperament test when horses are ridden, led, or running free, to evaluate the influence of a handler or rider on reactivity to novel stimuli. Results indicated that leading resulted in the lowest behavioral reactions, whereas the reactions under a rider were stronger. It could be assumed that a handler on the ground has a greater influence on the horse's behavior, controlling tactile cues, whereas the mounted rider is more limited in well-perceived forms of communication. During testing events when the horse was turned free, the reactive behavior expressed by each horse varied and remained specific to each horse. Therefore, it may be more accurate to evaluate a horse's temperament in relation to specific tasks that are most relevant in practice, as in when they are paired with a handler or rider.

Measuring Reactivity in Horses

Reactivity, or emotionality, in the horse is an increased state of arousal and may influence the usefulness of horses for specific tasks. (McCall et al., 2006) Reactivity is comprised of both behavioral and physiological components which must be correlated in an accurate test of reactivity to appropriately be measured (McCall et al., 2006). Broom (1991) agreed that evaluation of a stress response is best analyzed using a combination of both behavioral and physiological measures. This provides a stronger measurement of stress and avoids misleading conclusions.

To quantitatively and objectively measure reactivity in horses, McCall et al. (2006) assigned emotionality scores (ranging from 1-5) to each horse and compared scores to measures of reactivity to assess accurate correlations. Three different tests (novel stimuli, isolation, and runaway) were used to assess reactivity. Subjective emotionality scores based on an overall general impression were not good indicators of reactivity in horses. Additionally, objective field tests were not valid predictors of reactivity. Despite these conclusions, isolation tests as well as novel stimulus testing were valid tests of reactivity, as these tests displayed a strong correlation between the behavioral and physiological measurements.

Similarly, Borstel et al. (2011) compared behavioral (focusing on head-tossing and stumbling) and physiological (heart rate) observations on horses and riders during regular performance training and related them to different personality traits. Behavioral patterns such as head-tossing, stumbling, and heart rate variability were related to scores of personality traits. Care should be taken, however, when directly correlating undesirable behavior with poor personality in the horse (Borstel et al., 2011). Behavioral observations during riding in combination with special temperament tests were required to accurately link personality traits to behavior.

Continuing with the assessment of reactivity, Young et al. (2012) developed a scale of behavioral scores to measure stress levels in stabled domestic horses to assess their welfare. Behavioral (categories including movement of the whole body, tail, neck, ears, mouth, and head) and physiological (salivary cortisol concentrations and heart rate) measurements correlated to the behavioral scores from a scoring system assigned to each horse. Horses were ranked by four categories of stress (No stress, Low stress, Medium stress, and High stress) and assigned behavior scores ranging from 1-10 (1-2 No stress, 3-4 Low stress, 5-7 Medium stress, 8-10 High stress). All scores were linked to specific behavioral indicators. Young et al. (2012) believe using their scoring system reduces the need to measure various physiological parameters separately.

Horses possess the natural instinct of flight to escape threatening situations. When horses choose flight mode when exposed to sudden stimuli, this is a major contributor to horse-related injuries. Because of this, Noble et al. (2013) believe that reaction speed is valid in measuring reactivity in horses. Reaction speed may be useful to determine the effects of training to promote a decrease in reactivity, although further studies must be done to provide an accurate relationship between temperament and reactive behavior.

Calviello et al. (2016) proposed and validated the scale of composite measure score of behavior during daily handling techniques to evaluate reactivity in horses. Horses were scored in five categories (movement, position of ears and eyes, breathing, vocalization, and urination) on a scale of 1-4. Reactivity is specific to each individual because each animal expresses their own behavior. Despite this individuality, by combining the scores of the most relevant behaviors to explain horse reactivity, it may be possible to override the individual differences which in turn makes their scoring system stronger, compared to scoring systems by Young et al. (2012) and McCall et al. (2006).

Habituation in Horses

Survivability of horses in the wild is highly dependent on fear reactions to avoid sources of danger, allowing their lifespan to increase. Despite this instinctive reaction, horses are conscious to which stimuli they react to as this reaction disposes energy quickly. Manning and Dawkins (1998) define habituation as "a simple form of learning, which is relatively long-lasting and 'stimulus-specific', i.e. only the stimuli which are repeated without reinforcement are affected – the animal remains alert to others". According to Rankin et al. (2009) habituation is defined as "a waning of responsiveness towards neutral stimuli, which do not result in any reinforcement (negative or positive) and functions to reduce responses to innocuous stimuli".

Christensen et al. (2008) investigated the extent to which horses habituate to six differently colored and shaped novel objects in reducing reactivity to fear testing, involving visual and tactile stimulation. Horses demonstrated generalization between similarly colored objects and stationary objects or varying shape. There was a lack of generalization between the same objects with different colors in a voluntary test situation. Horses previously habituated to six visual objects of varying color and shape did not respond less in a fear-test involving visual and tactile stimulation. These results support the thought that horses habituate to specific stimuli and a large amount of similarity between objects is required for generalization (Christensen et al., 2008).

In a new series of testing events, Christensen et al. (2011) continued to explore the ability of horses to generalize between objects during habituation. Horses demonstrated both object recognition and generalization. Horses elicited less reactive behaviors when exposed to a novel object of a new shape and color. These results differed from their previous study (Christensen et al., 2008). This difference may have been caused by presenting novel stimuli in the same environment, allowing the horses to habituate to the environment, in turn no longer eliciting frightening perceptions to new stimuli (Christensen et al., 2011).

Leiner and Fendt (2011) investigated behavioral (facial signals, vocalization, and avoidance behavior) and physiological (heart rate) fear responses in horses and how reactivity changes after repeated exposure to two novel stimuli (blue and white umbrella and orange, plastic tarp). Horses expressed a wide range of behavioral signs to fear and the number of these responses was correlated to the response of heart rate. The fear response to the umbrella was reduced, whereas the fear response to the tarp was not. These results support those by Christensen et al. (2008, 2011) concluding the effect of habituation training in horses is object specific. Additionally, fear responses towards a non-habituated object are still present.

CHAPTER II: MATERIALS AND METHODS

Horses are natural prey animals and their greatest means of defense to novel stimuli is to react and run away. This natural reaction can be problematic and must be managed in horses used for different types of work. The purpose of the current study was to identify patterns of behavior in horses trained for Mounted Patrol, Equine Assisted Activities and Therapies, and Show. Identifying patterns of reactivity in horses used for different types of work supports appropriate selection of horses used for specific jobs.

Animals

The protocol was approved by the Institutional Animal Care and Use Committee (Protocol #17-2003, Appendix). Eighteen healthy horses previously trained for use in one of three areas, Mounted Patrol, Equine Assisted Activities and Therapies (EAAT), or Show, were evenly assigned to one of three groups (Patrol, EAAT, or Show). Six Percheron Drafts (aged 10 ± 3 yr including three mares and three geldings) were obtained from Rutherford County Mounted Patrol. The remaining twelve horses were obtained from Middle Tennessee State University Horse Science Center. Six horses (aged 14 ± 6 yr including three mares and three geldings) were assigned to the EAAT group. Six horses (aged 13 ± 2 yr including two mares and four geldings) were assigned to the Show group. Breeds for these twelve horses included two Tennessee Walking Horses, six Quarter Horse, one Thoroughbred, two Warmbloods, and one Arabian. The Mounted Patrol horses were intended to serve as a positive control as they had all previously been exposed to the stimuli used.

Experimental Design

All testing occurred in an 18 m diameter round pen housed inside the Tennessee Miller Coliseum in order to negate any effects of weather, sunlight or shadows. Three novel stimuli were used during testing including a fog machine (Amscan Inc., Elmsford, NY), inflatable air dancer (AirDancers®, San Francisco, CA), and paintball gun (Cronus, Tippmann Sports Inc., Fort Wayne, IN). The fog machine was placed at ground level alongside the perimeter of the round pen, providing both visual (fog) and auditory (air hissing) components. The inflatable air dancer (5.2 m tall, 0.61 m wide) was placed 24.7 m away from the center of the round pen and 6.4 m above ground level, providing both visual (inflation and movement of the air dancer) and auditory (air compressor motor) components. The paintball gun was placed 0.61 m from the perimeter of the round pen, and blank rounds were fired away from the direction of the testing area, providing only an auditory component.

All horses were fitted with a heart rate monitor (Polar RC3 GPS, Polar Electro Inc., Lake Success, NY) and Fitbit One (Fitbit Inc., San Francisco, CA) to assess reactivity. Each horse was individually turned loose inside the round pen and given a 30 s period to adjust to the testing environment. Following the adjustment period the horses were exposed to one of the three stimuli for 30 s to measure initial reactivity. Exposure to each stimulus was temporarily ceased for 60 s. Behavioral observations were continually observed and collected. Exposure to each stimulus resumed at 120 s and the time it took for the horse to become habituated to the stimulus was measured when there was a drop in heart rate or no signs of reactive behavior. In the event that a horse reacted adversely to any of the stimuli in a manner that was deemed unsafe for the horse, or if a horse did not habituate prior to 3 minutes, all testing was ceased, the horse was immediately taken out of the pen and led back to their stall where their resting period began.

After each horse had been exposed to the specific stimulus, and data had been collected for that horse, they then proceeded to their individual resting period, which lasted at least 5 minutes for each horse. This period of time was monitored and controlled by the designated horse handler. All horses in which data was not currently being collected remained in stalls within MTSU's Horse Science barn.

These procedures were repeated for each stimulus per each horse. Heart rate was consistently recorded every 10 s and the Fitbit One step count was recorded by difference in the values recorded immediately prior to entering and after exiting the round pen. In between testing events, all horses remained in 12 x 12 m stalls and were kept under the supervision of the designated horse handlers.

Statistical Analysis

Data were tested for normality using a Shapiro-Wilk statistic. The fixed main effects of stimulus and group on heart rate, habituation time and step count were analyzed using a mixed model with repeated measures (SAS Ver. 9.25, SAS Inst., Cary, NC), with horse as the subject, and time as the repeated effect. Statistical significance was considered at $P < 0.05$ and trends were considered at $0.05 < P < 0.10$.

CHAPTER III: RESULTS

The Patrol horses elicited greater HR than EAAT (*P* = 0.023) and Show (*P* = 0.012) horses with no difference between EAAT and Show (*P* = 0.76) horses when exposed to the fog machine (Figure 1). There was no difference in HR between work types when exposed to the air dancer (Figure 2). The Patrol horses showed greater HR than EAAT (*P* = 0.046) and Show (*P* = 0.027) horses with no difference between EAAT and Show (*P* = 0.79) horses when exposed to the paintball gun (Figure 3). There was no difference in habituation between work types when exposed to the fog machine (Figure 4)or paintball gun (Figure 5). EAAT horses tended to reach habituation quicker than Show horses (*P* = 0.073) when exposed to the air dancer (Figure 6). There was no difference in step count between work types when exposed to the air dancer (Figure 7) or paintball gun (Figure 8). Patrol horses elicited a greater step count than EAAT (*P* = 0.017) and Show (*P* = 0.014) horses with no difference between EAAT and Show (*P* = 0.71) horses when exposed to the fog machine (Figure 9). These results agree with previous studies while also showing that type of work affects reactivity when exposed to novel stimuli. Understanding how type of work affects horse reactivity is important to professionals engaged in patrol work, EAAT, and horse shows.

CHAPTER IV: DISCUSSION

The results of this study indicate that type of work influenced reactivity when horses were exposed to novel stimuli. Although the mounted patrol horses were intended to serve as a positive control group, as they had all previously been exposed to the three stimuli used, their heart rates were greater than EAAT and Show horses when exposed to the fog machine and paintball gun. Previous research (Munsters et al., 2013) evaluating the stress levels of horses used in mounted patrol training concluded that the interaction between horse and rider does have an effect on reactivity, as horses elicited higher heart rates when not being ridden compared to horses that were paired with a rider. It is suspected that the mounted patrol horses in this study elicited greater heart rates due to the fact that they were without their riders during all testing events. Minero et al. (2006) evaluated reactivity of therapy horses compared to jumping horses, by exposing them to novel stimuli, and concluded that horses used in therapy work elicited higher heart rates compared to jumping horses. The current study found no differences between the EAAT and Show horses in any of the variables when assessing reactivity. Lastly, there were no differences in this study between any of the groups focusing on habituation time. Christensen et al. (2008, 2011) evaluated the influence of object shape and color to reactivity in horses. These horses were able to generalize between objects of similar shape but different color, whereas there was a lack of generalization between objects of different shapes but the same color. Leiner and Fendt (2011) investigated fear responses by exposing horses to two novel stimuli (blue and white umbrella and orange, plastic tarp). Following habituation training, fear responses were still present in horses when exposed to the tarp, but not to the umbrella. It is suspected that the stimuli used in the current study had similar components, supporting these results.

CHAPTER V: CONCLUSION

Training for specific types of work, particularly jobs such as mounted patrol, equine-assisted activities and therapies, and showing, influences reactive behavior to a novel stimulus in horses. Heart rate is affected by type of work to a novel stimulus, but the time to habituate to that stimulus is not. Reactivity remains stimulus specific as each stimulus elicited different reactive responses. Understanding how type of work and different stimuli affect horse reactivity is important to professionals engaged in mounted patrol work, equine-assisted activities and therapies, and horse shows. Future research in equine reactivity and habituation may further understanding in this area and allow for improved selection of horses for specific types of work based on reactivity to natural stimuli.

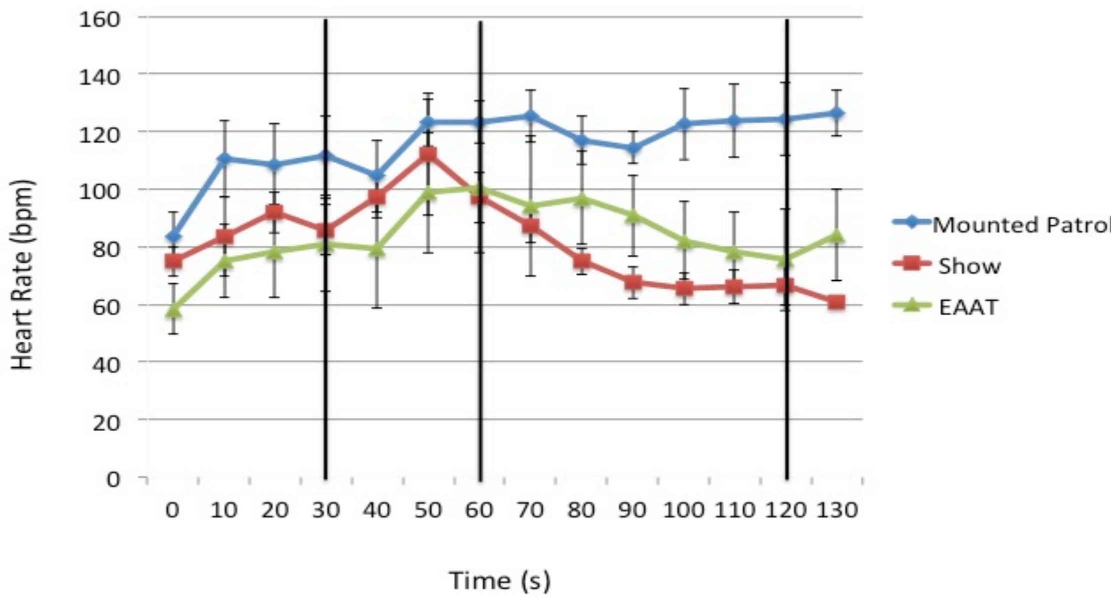

Figure 1. Average heart rates (HR) for Patrol, Show, and equine-assisted activities and therapies (EAAT) horses when exposed to the fog machine. Vertical lines denote Left to Right: initial reaction to the round pen, 30 s exposure to the fog machine, 60 s recovery, then exposure to the fog machine to reach habituation. The Patrol horses elicited greater HR than EAAT (P = 0.023) and Show (P = 0.012) horses with no difference between EAAT and Show (P = 0.76) horses.

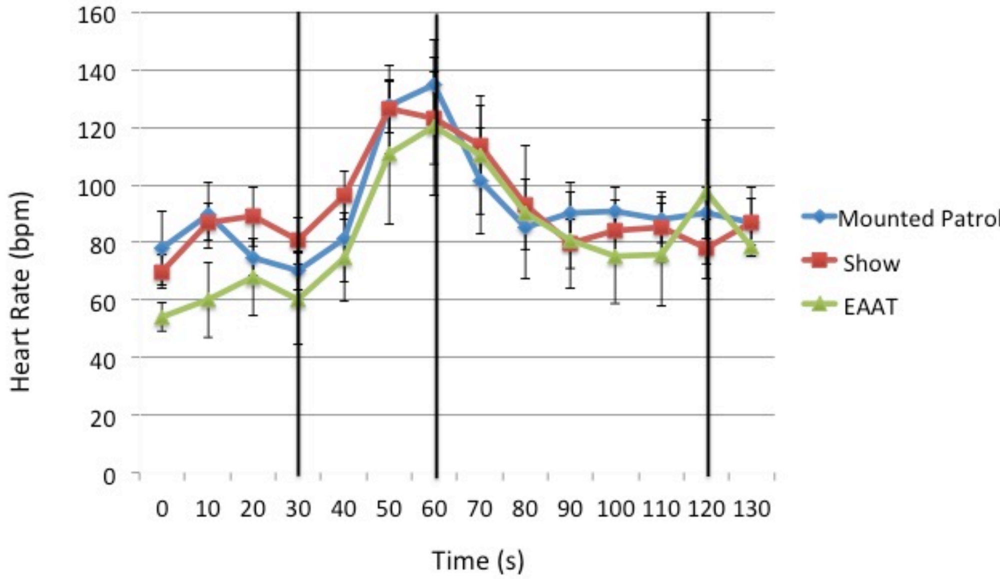

Figure 2. Average heart rates (HR) for Patrol, Show, and equine-assisted activities and therapies (EAAT) horses when exposed to the air dancer. Vertical lines denote Left to Right: initial reaction to the round pen, 30 s exposure to the air dancer, 60 s recovery, then exposure to the air dancer to reach habituation. There was no difference in HR between type of work.

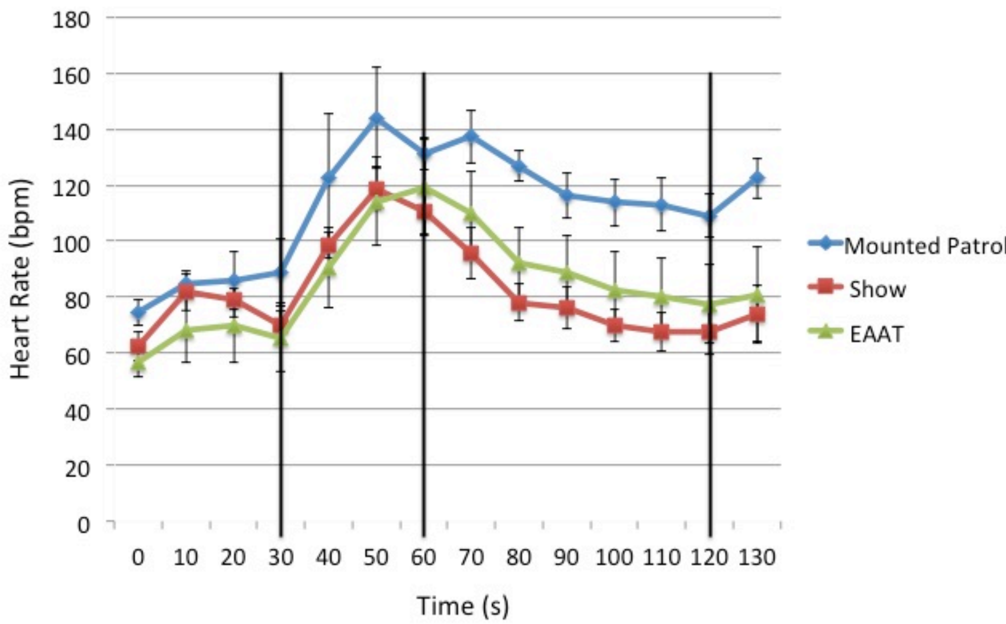

Figure 3. Average heart rates (HR) for Patrol, Show, and equine-assisted activities and therapies (EAAT) horses when exposed to the paintball gun. Vertical lines denote Left to Right: initial reaction to the round pen, 30 s exposure to the paintball gun, 60 s recovery, then exposure to the paintball gun to reach habituation. The Patrol horses showed greater HR than EAAT ($P = 0.046$) and Show ($P = 0.027$) horses with no difference between EAAT and Show ($P = 0.79$) horses.

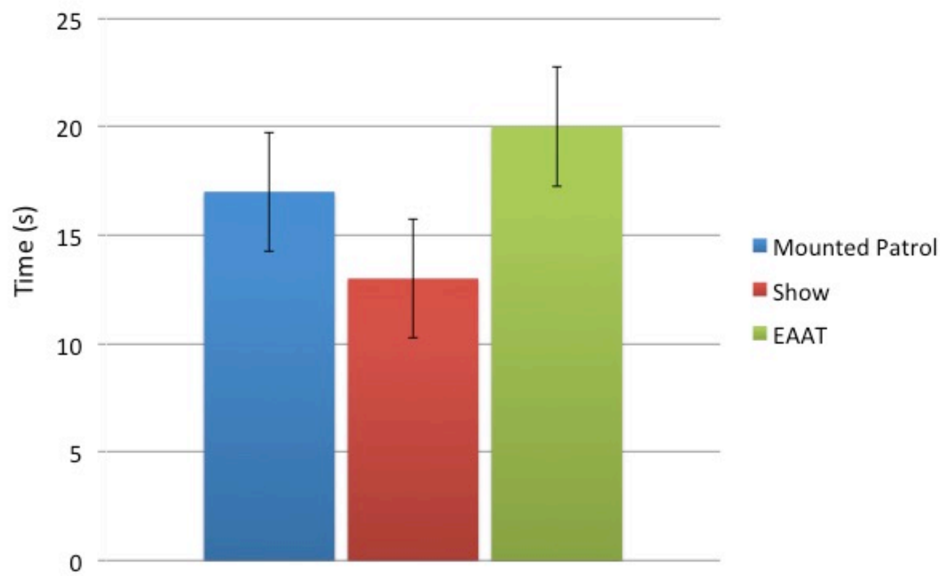

Figure 4. Time (s) to reach habituation for Patrol, Show, and equine-assisted activities and therapies (EAAT) horses when exposed to the fog machine. There was no difference in habituation time between type of work.

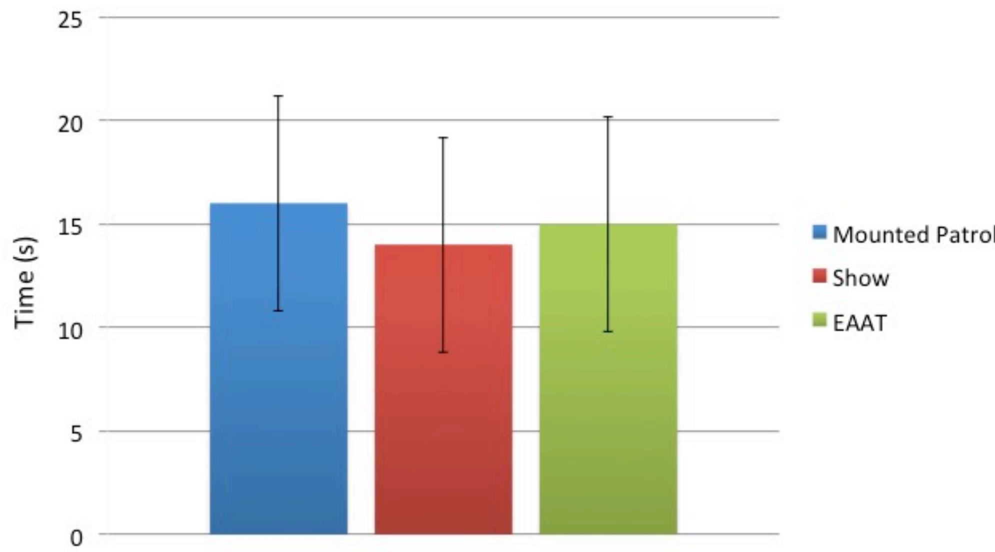

Figure 5. Time (s) to reach habituation for Patrol, Show, and equine-assisted activities and therapies (EAAT) horses when exposed to the paintball gun. There was no difference in habituation time between type of work.

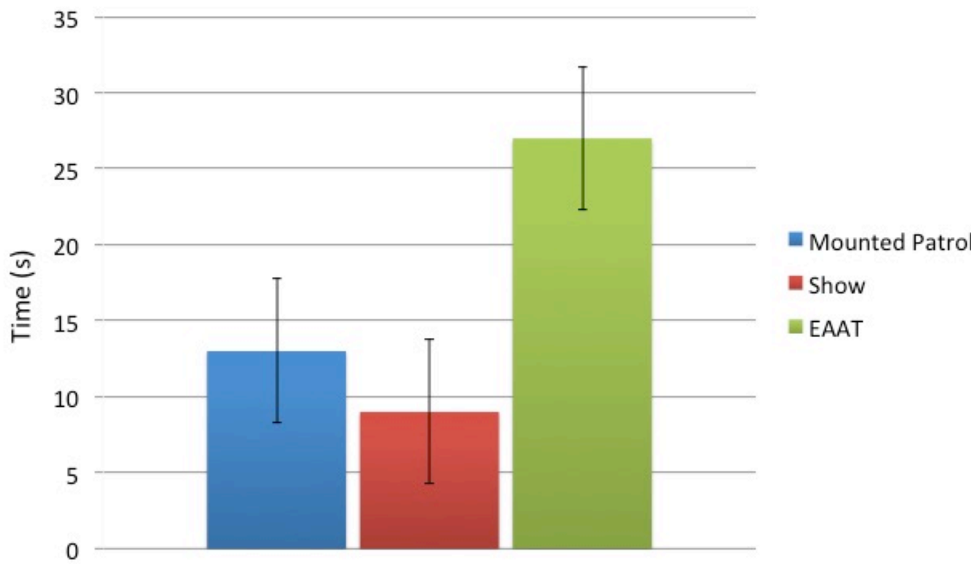

Figure 6. Time (s) to reach habituation for Patrol, Show, and equine-assisted activities and therapies (EAAT) horses when exposed to the air dancer. EAAT horses tended to reach habituation quicker than Show horses (*P* = 0.073).

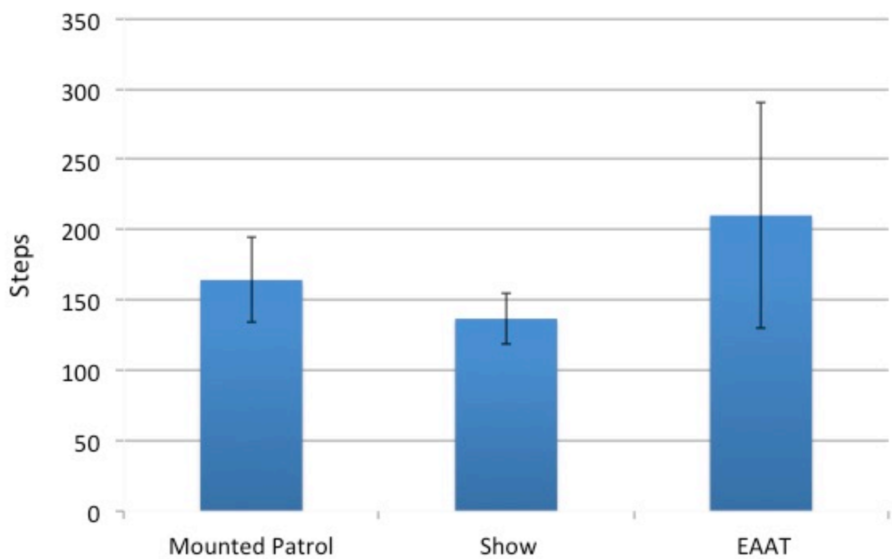

Figure 7. Fitbit One step count for Patrol, Show, and equine-assisted activities and therapies (EAAT) horses when exposed to the air dancer. There was no difference in step count between type of work.

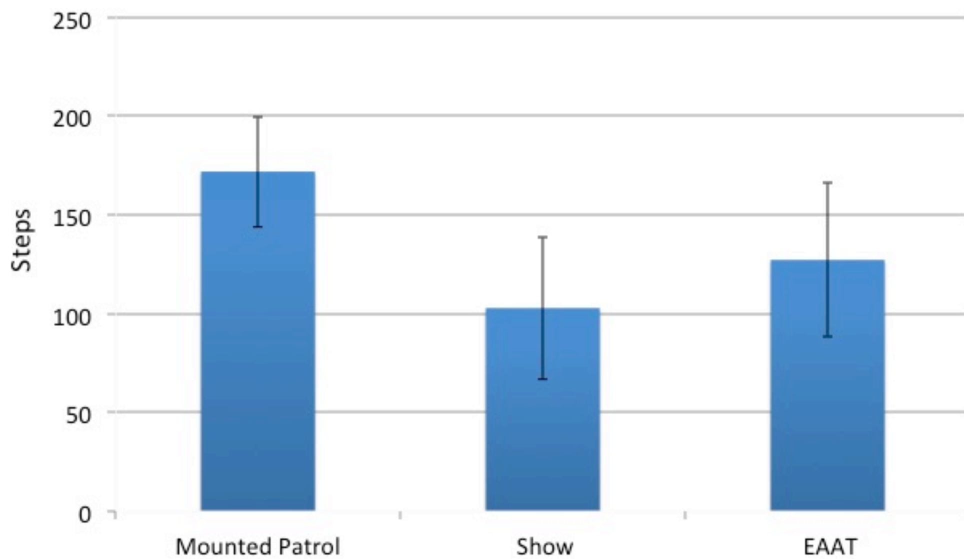

Figure 8. Fitbit One step count for Patrol, Show, and equine-assisted activities and therapies (EAAT) horses when exposed to the paintball gun. There was no difference in step count between type of work.

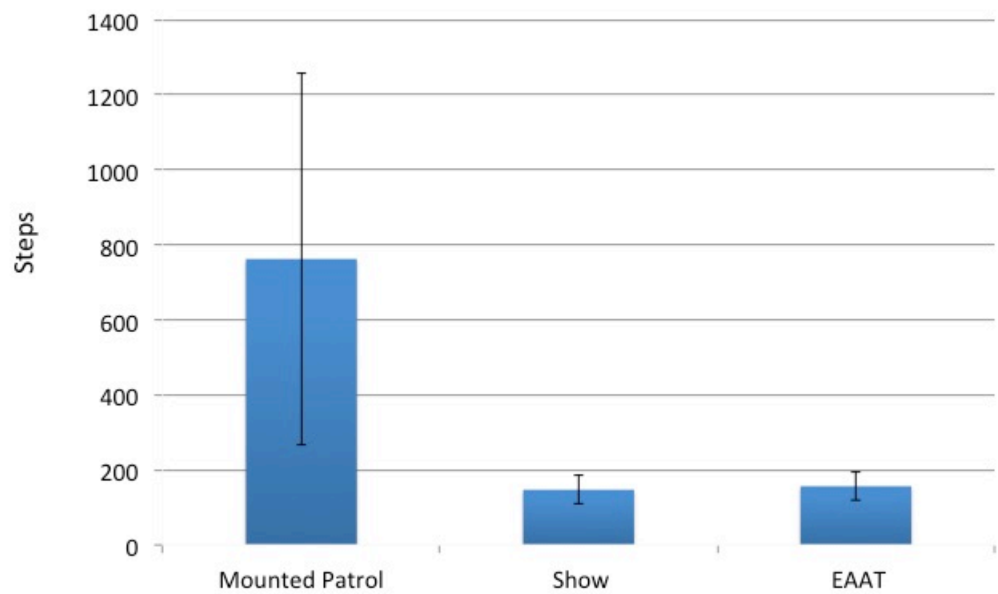

Figure 9. Fitbit One step count for Patrol, Show, and equine-assisted activities and therapies (EAAT) horses when exposed to the fog machine. Patrol horses elicited a greater step count than EAAT (P = 0.017) and Show (P = 0.014) horses with no difference between EAAT and Show (P = 0.71) horses.

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APPENDICES

APPENDIX A: IACUC Approval

IACUC
INSTITUTIONAL ANIMAL CARE and USE COMMITEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

MIDDLE TENNESSEE STATE UNIVERSITY

IACUCN001: PROTOCOL APPROVAL NOTICE

Thursday, November 17, 2016

Investigator(s): Rhonda Hoffman (PI), Anne Brzezicki (co-FA), Chloe Wires (student) and Erica Macon (student)
Investigator Email(s): Rhonda.hoffman@mtsu.edu; anne.brzezicki@mtsu.edu; ccw4l@mtmail.mtsu.edu; elm4v@mtmail.mtsu.edu
Department/Unit: ABAS

Protocol ID: **17-2003**
Protocol Title: ***Exploring horse reactivity and habituation across work types***

Dear Investigator(s),

The MTSU Institutional Animal Care and Use Committee has reviewed the animal use proposal identified above under the ***Designated Member Review (DMR) mechanism*** and has approved your protocol in accordance with PHS policy. A summary of the IACUC action(s) and other particulars of this this protocol is tabulated as below:

IACUC Action	**APPROVED for one year from the date of this notification**			
Date of Expiration	**11/30/2017**			
Number of Animals	24 (TWENTY FOUR)			
Approved Species	**Equine (16 from MTSU HSC and 8 from Rutherford Co Mounted Patrol**			
Category Subclassifications	☐ **Teaching**	☒ **Research**		
	☐ Classroom	☐ Laboratory	☒ Field Research	☐ Field Study
	☐ Laboratory	☒ Handling/Manipulation		☐ Observation
	Comment: Graduate student research			
Approved Site(s)	Horse Science Center			
Restrictions	**Satisfy DMR requirements; MUST complete annual continuing review as shown below; Students MUST complete annual health screening.**			
Comments	NONE			
Amendments	***Date***	***Post-approval Amendments***		
		NONE		

This approval is effective for three (3) years from the date of this notice. This protocol **expires on 11/30/2019** The investigator(s) MUST file a Progress Report annually regarding the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be approved by the IACUC prior to **11/30/2017** for this protocol to be active for its full term. Once a protocol has expired, it cannot be continued and the investigators must request a fresh protocol.

Continuing Review Schedule:

Reporting Period	Requisition Deadline	
First year report	10/31/2017	INCOMPLETE
Second year report	10/31/2018	INCOMPLETE

Final report	10/31/2019	INCOMPLETE

MTSU Policy defines an investigator as someone who has contact with live or dead animals for research or teaching purposes. Anyone meeting this definition must be listed on your protocol and must complete appropriate training through the CITI program. Addition of investigators requires submission of an Addendum request to the Office of Research Compliance.

The IACUC must be notified of any proposed protocol changes prior to their implementation. Unanticipated harms to subjects or adverse events must be reported within 48 hours to the Office of Compliance at (615) 494-8918 and by email – compliance@mtsu.edu.

All records pertaining to the animal care be retained by the MTSU faculty in charge for at least three (3) years AFTER the study is completed. Be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually. Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

Sincerely,

Compliance Office
(On behalf of IACUC)
Middle Tennessee State University
Tel: 615 494 8918
Email: iacuc_information@mtsu.edu (for questions) and
Iacuc_submissions@mtsu.edu (for sending documents)