THESIS

EVALUATING THE REACTION TO A FAMILIAR COMPLEX ROTATED OBJECT IN DOMESTIC HORSES (EQUUS CABALLUS)

Submitted by

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ABSTRACT

EVALUATING THE REACTION TO A FAMILIAR COMPLEX ROTATED OBJECT IN DOMESTIC HORSES (EQUUS CABALLUS)

It is dangerous for both riders and horses when a horse suddenly startles. Sometimes horses do this in familiar environments with a possible cause being that familiar objects may look different when rotated. The purpose of this study was to determine whether horses that had been habituated to a complex object (children's plastic playset) would react to the object as novel when it was rotated 90 degrees. Twenty young horses were led past one side of the playset 15 times by a handler. Horses in the rotated group were led past the rotated playset 15 times, while the control group continued to be led past the playset in its original position. The behavioral signs observed and analyzed were ears focused on the object, nostril flares, neck raising, snort, avoid by stopping, avoid by moving feet sideways, and avoid by flight. The most common reactions observed were ears focused on the object, nostril flares and neck raising. Reactions were mild because the horses used were safe to lead and all procedures were done at a walk. When the playset was rotated, the behavioral signs observed were similar to behaviors exhibited during the first exposure to the playset. A two- sample t test was performed on the reactivity scores that compared the number of behavioral signs present on pass 1 compared to pass 16 by the rotated object. The horses in the rotated group reacted to the rotated orientation similarly to the first exposure ($p = 0.0014$, $a < 0.05$). Two-sample t-tests were conducted for corresponding passes 2-15 for the novel object to rotated object. There was little consistent association for the corresponding passes, showing the effect of the unpredictability of the horse. Awareness of

potential reactions to changes in the orientation of previously familiar objects can help keep the handler safer. Horses' reaction to a rotated orientation of a familiar object and reduction in reaction over time will be similar to their original exposure.

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CHAPTER 1: LITERATURE REVIEW

Introduction

Horses are prey animals and exhibit behaviors that help them adapt and survive in their environment. Fear reactions to perceived danger are essential for survival. These reactions are often referred to as spooking and they have the potential to be dangerous to the horse, handler and rider. Spooking consists of avoidance reactions that include suddenly moving away or running away from the perceived danger. To prevent horses or other prey species from having constant flight reactions, they must be able to filter out unimportant stimuli (Hanggi, 2005). Horses can learn what is safe and what is perceived as dangerous. When presented with novel objects in their environment, horses will slowly become accustomed to the novel object through repeated exposure. This is known as habituation (Cooper, 1998) and is seen in other livestock animals as well (Grandin and Deesing, 2014). Horses use habituation and many other different learning tools to process and survive in their natural environment. These learning tools include, but are not limited to; habituation, discrimination, generalization, categorization and memory. These learning tools are often studied in horses and many other species to provide insight into perception and cognition. There is little research on how equine visual perception relates to learning and cognition, when compared to other species (Nichol, 2002).

Anecdotally, many people in the equine industry have observed that horses will react, sometimes violently, to a familiar object when they see it in a different orientation. This reaction may be due to the horse not recognizing the object when it is in a new position. This concept has been evaluated by Hanggi (2010) where discrimination and food reinforcement, or positive reinforcement, was used to teach the horses to voluntarily walk towards the ‘correct’ object.

These authors found that horses were able to discriminate between several different orientations of the same object after learning through positive reinforcement (Hanggi 2010). This research was helpful to evaluate how horses learn when positive reinforcement was used. It does not provide insight into how horses might behave when being led by a handler or ridden towards either a novel object or an object in a new orientation. Additionally, horses may behave differently if allowed to voluntarily approach as opposed to a non-voluntary approach, such as being led.

The interaction between horses and humans and the impact it has on that relationship is often referred to as the horse-human relationship. The human-horse relationship has been shown to have an impact on how the horse reacts to stimuli (Gorecka et al, 2007). Borstel et al (2011) found that when comparing reactivity in temperament tests of horses free-running, being led and being ridden, horses being led and ridden reacted differently than free-running horses. The prevalence of accidents and injuries in the horse industry is high, with spooking being a common cause of accidents (Camargo et al., 2018). This risk of handling and riding horses is acknowledged by the equine community due to the unpredictable nature of the horse. (Thompson et al., 2015). While this risk is acknowledged, there should be more efforts to help handlers understand the horse and be better prepared to avoid accidents.

Further research is needed to evaluate how the human-horse relationship changes the way the horse learns and perceives their environment. This literature review will serve to explore the ways in which horses react to novel objects and novel orientations of previously familiar objects. Additionally, this review will explore how horses behavior changes between different learning tests and the presence or absence of a handler.

Equine Adaptive Behavior

Horses have innate adaptive behavior for survival as prey animals. The way horses adapt to their environment is influenced by their vision. Horses have one of the largest eyes of land mammals and exceptional distance vision, and they use vision to obtain information about their environment (McGreevy, 2012). Eye placement on the side of their head provides the advantage of being able to see all around themselves monocularly (sight out of one eye). The disadvantage of their eye placement is limited binocular vision (sight with both eyes) in front of and behind them. The horses eye placement and vision enables them to widely survey their surroundings and be alert to subtle changes in their environment.

When horses are faced with perceived dangerous stimuli they either will fight, flight (McGreevy, 2012) or freeze (Smith et al., 2018). These adaptive behaviors are seen in both wild and domestic horses. It is rare to see a fight response in domestic horses unless they are exposed to aversive or continued negative experiences. Some adaptive behaviors in horses can be lessened over time and exposure, such as flight reactions to perceived danger. One example of this adaptive behavior is exposure of foals to repeated handling. Overtime, this will decrease their heart rate and improve ease of handling, compared to unhandled 'forest raised' horses (Jezierski, 1999) . When horses are young, they often flee away from humans because they are novel. Through exposure and repeated handling this behavior decreases over time (Jezierski, 1999). These flight reactions may occur in older horses exposed to novel objects, loud noises, and fast-moving things. Similar to handling as a foal, repeated exposure to being handled by humans lessens this flight reaction.

Habituation is a non-associative learning tool observed in animals. Habituation is useful as it enables horses to filter out non-threatening stimuli so they can focus on what the horse perceives as important or life-threatening (Hanggi, 2005). Habituation is defined as a lessened reaction or reduced avoidance to a previously novel stimulus over repeated exposure (Cooper, 1998). Habituation has been shown to reduce fearful reactions as well as physiological signs of fear by repeated exposure to novel objects (Leiner and Fendt, 2011). In a study by Leiner and Fendt (2011) horses were exposed to an umbrella and a tarp on day 1, then the horses were habituated to only the umbrella through repeated exposure for the following 5 days. On day 7, the horses were exposed to the umbrella and tarp together again to show that the horses expressed a difference in reaction between the two objects. The horses reacted more to the tarp. This study shows the effects of habituation over repeated exposure to a novel object (Leiner and Fendt, 2011). Horses use habituation to understand and react to the world around them, in their natural environment or training sessions.

Novel Object Tests

The horse's response to novel objects is an important tool for survival and avoidance of potentially dangerous novel stimuli. Avoidance behavior helps the horse survive in their natural environment by keeping them away from something that has the potential to harm them. As mentioned earlier, habituation is a learning tool used to help horses to determine dangerous or not-dangerous novel stimuli. Novel object tests are a common tool in research to evaluate temperament, reactivity, and emotionality (Vissers et al., 2002).

Exposure to novel objects has been shown to induce reactive behaviors in horses, such as head/neck raising and avoidance movement (horses: Leiner and Fendt, 2011; Christensen et al., 2005; Visser et al., 2002). These reactive behaviors caused by exposure to novel object have also been seen in cattle (Grandin, 1997) and pigs (Dalmau, 2009). Researchers often use behavioral signs to evaluate the flight response to a novel object, but the behaviors analyzed were different between studies. Leiner and Fendt (2011) evaluated behavioral signs when horses were exposed to novel objects. The behavioral signs recorded by Leiner and Fendt (2011) were

> "ears pointing towards the object plus focusing, elongation of the upper lip, tense neck muscles (elevated neck), snorting (short powerful exhalation from nostrils), snuffling, avoidance behavior leaning backwards, avoidance behavior with evasive movements (steps to the side), avoidance behavior with evasive movements (steps) back and flight behavior (jumping away in a sudden movement, typically followed by trotting/galloping)".

Christensen et al. (2008, 2011) used the following behaviors to evaluate reaction to a novel object; eating, sniff object, investigate object, alert towards object, head lift during eating (only observed in Christensen et al., 2008), defecation, snort, paw bout. In a different study by J.W. Christensen, only 5 behaviors were measured in a reactivity scale; none, head up , alert, moving away or flight (Christensen et al., 2006).

Heart rate monitors have been used as a tool to evaluate a horse's physiological response to novel objects or other stimuli. A rise in heart rate when a horse first sees a stimuli can provide evidence that the behaviors shown are potentially associated with fear and the novelty of the object.

Heart rate monitors paired with behavioral responses can be a reliable way to measure flight responses. One study showed that heart rate would change when no behavioral responses were shown (Christensen et al., 2006). Another study showed that heart rate increased at first sight of the object, shortly before avoidance behavior occured (Leiner and Fendt, 2011). The variation in heart rate as compared to behavioral responses makes heart rate a difficult tool to use on its own without also measuring behavior responses. Behavioral responses and flight reactions have been shown to be independent of heart rate. Behavioral responses have been a major focus in research because behavioral reactions to stimuli may cause dangerous situations in training and handling.

Novel object tests are most often done with the horse being allowed to voluntarily approach the novel stimuli. When using a voluntary approach, there is no handler leading the horse. The horse is free, and latency to eat (time it takes for the horse to eat at the object) is often observed and recorded. In other species, such as, pigs and cattle, latency to touch instead of latency to eat is more often used in novel object tests. With latency to touch, researchers record how long it took for the animal to touch the object with no food present (Hemsworth et al., 1996; Herskin et al., 2004). In research with horses, latency to eat is often used. In preparation for the study discussed in Chapter 2, a preliminary study was done with 3 Arabian horses between the ages of 12-15. They were allowed to free roam in a test arena with a complex novel object (child's playset). This preliminary study used latency to touch. The three horses voluntarily approached the object and showed different latency to touch. They showed no reaction even at the first exposure to the object. Voluntary approach is often observed when horses (and other livestock animals) interact with novelty in their home environment (Grandin, 1997). Their behavior may be different when a horse is exposed to a novel object when a handler is leading them. This anecdotal observation prompts discussion on how voluntary vs non-voluntary approach to a novel object have different effects on horse behavior.

Learning Tests

There are many different learning tests that can give insight into equine perception and cognition. Discrimination learning is described as how horse behaviors change due to cues in the environment. This is often studied by having the subject complete a task by using spatial, visual or auditory cues (Kratzer et al., 1977; Marinier and Alexander, 1994). The horses are judged on how they perform by their ability to choose the "correct" task. The "correct" task, "correct" decision is then rewarded with some sort of reinforcement, usually positive in the form of a treat.

Flannery (1997) used positive reinforcement in the form of carrots and a clicker to shape the horses behavior to touch “correct” cards in displayed in various ways on a wall. The cards all had either a circle, X, star or square. The horses were able to discriminate between the different cards in later trials (Flannery, 1997). A later study found that when buckets with square, stripe and solid patterns were arranged in different positions on a wall, foals tend to discriminate better spatially (by location) than by pattern or color (Hothersall et al., 2010). Horses have also been shown to discriminate objects by placement and by size (Hanggi, 2003; Mal et al., 1993). These tests give us insight into how horses discriminate between stimuli.

Generalization uses knowledge from previously discriminated stimuli to apply to other similar stimuli. The concept of generalization by size or shape is often shown in situations learned by discrimination (Christensen et al., 2008; Dougherty and Lewis, 1991; Flannery, 1997; Hanggi, 2003). In the study mentioned above, horses were still able to identify “correct” cards when the cards were arranged in a different positions than the positions in the original trial (Flannery, 1997). In another study, horses were trained to a tactile stimulus on their back and they were able to respond correctly to similar stimuli placed on a different place on their back (Dougherty and Lewis, 1993). The tactile stimuli used in their study was a belt that was laid across the horse’s spine with small cylinders to lightly tap the horse in different places on their back. The researcher trained the horse to an original stimuli of just one cylinder tapping the horse’s back in one position. The horses were able to respond correctly to generalized stimuli the closer the stimuli was to the originally trained stimuli, but has less “correct” responses as the stimuli moved farther away on their back (Dougherty and Lewis, 1993). Horses are able to generalize stimuli with similar qualities but seem to have a harder time as the stimuli becomes more different from the original. Horses also have the ability to generalize novel stimuli they have been previously habituated to (McLean, 2003). There is limited knowledge about how generalization is related to habituation in horses (Nichol, 2002; Murphy and Arkins, 2007).

Categorization is a learning behavior in horses where they can place generalized stimuli into categories based on their past experiences. Shape and color have been observed as factors that affect how horses categorize objects (Hanggi, 1999). Hanggi (1999) states that categorization is a useful tool for survival in horses because not every stimulus will be exactly the same. The ability to categorize stimuli is a helpful tool in the horses natural environment. Anecdotally, animals can also categorize experiences by negative stimuli. For example, if an animal has a negative experience with someone wearing a hat, a different person wearing the same hat could cause the animal to react. In this example, the animal has categorized people wearing hats as a negative experience.

Horses have been shown to remember how to solve mazes in times as short as a week (Mariner and Alexander, 1994) or even as long as a month after (Wolff and Hausberger, 1996). Mariner and Alexander (1994) conducted a study where nine horses learned and completed Maze A and B until they reached a specified criterion of success, set by the researchers. The same horses were then run through the same procedure one week later and two months later. All horses were able to remember and complete the maze correctly, but the time to complete the maze showed variety in how well the horse's memory of the maze was (Mariner and Alexander, 1994). Additionally, discrimination learning between objects has been shown to be retained as long as seven years (Hanggi and Ingersoll, 2009).

While horses can remember the same mazes for long periods of time, horses (Sappington et al., 1997; McCall et al., 2003), as well as cattle (Grandin et al., 1994), have trouble completing modified or reversed versions of previously learned tasks.

Learning tools such as, habituation, discrimination, generalization, categorization and memory assist horses with processing and understanding the world around them for survival. Using these learning tools as tests in research can inform how the horse perceives the world around them.

Human-Horse Interaction

The human-horse relationship has an impact on horse behavior. Horses are livestock species that were originally used for food production but changed over time to serve humans in a more recreational and working manner (McGreevy, 2012). This change in how we use horses changed the horse-human relationship. In the modern United States, horses are seen as athletes and pets, rather than food. Humans handle and train horses very differently than cattle, pigs or sheep. While many livestock animals behave in a similar way as prey animals, their difference in handling and use should change how we study their behavior.

The human-horse relationship has been shown to have an impact on how the horse reacts to stimuli. Borstel et al (2011) found that when comparing reactivity in temperament tests of horses free-running, being led and being ridden, horses being led and ridden reacted differently than horses free-running. Sondergaard and Halekoh (2003) used young Warmblood horses grouped by housing (individual vs group housing) and handling (handled or not-handled). The authors found that horses that had been handled showed a change in reaction to novel environments. They also found that horses accustomed to being handled showed less reaction to novelty than those that had less handling (Sondergaard and Halekoh, 2003). This difference in behavior between the different handling methods is important to consider. In the industry, when horses are being used, they are handled or ridden. Except when the horse is in their pasture or stall, it is very rare that horses are free to roam without interaction with handlers. When horses are being handled or ridden, injuries and accidents are very prevalent. Additionally, A horse's sudden flight reaction can be dangerous and can cause accidents that risk the safety of the horse and human (Angoules et al., 2018). Spooking can be a common cause of accidents (Camargo et al., 2018). The risk of spooking and accidents has been shown to increase when the handler is mounted and as speed increases (Hawson et al., 2010).

Horses that have been handled often have shown less reactivity when being handled (Vissers et al., 2002). Horses that have more of an opportunity to be habituated to humans and handled by humans tend to be less reactive. Older horses may be less reactive. They have had more time with humans throughout their life and show less reactivity and less emotionality (Munsters et al., 2012). Additionally, Gorecka et al. (2007) found that handlers had an effect on how horses react to novelty in the environment. The authors found an increased willingness to approach the novel object (open umbrella) with the horses being led by a handler as opposed to free, but no difference in startle reaction (Gorecka et al. 2007). This leads to a discussion on the impact of the handler when both conducting research and making conclusions from research findings. Vissers et al. (2002) evaluated young Warmblood horses in novel object and handling tests. The horses were grouped by time handled and trained, with some with less than five months of training and more than five months. The handler has been shown to mask some natural horse behavior (Vissers et al., 2002), but accidents and injuries when horses are being handled and ridden are still prevalent.

Discussion

Learning tests where the horses free to roam are helpful for providing insight into natural horse behavior. These learning tests also allow researchers to have better knowledge of equine visual perception and cognition. Research conducted by allowing the horse to be free often use latency to eat to evaluate how the horse learns. Using food as reinforcement in training is similar to using latency to eat in research. It is important to note though, that most trainers do not use food as reinforcement in their training. Training methodologies are worth further exploration when looking at equine perception of novel objects.

Accidents and injuries are very common in the horse industry. These accidents often lead to injury of the horse, human or both. This risk of accidents increases when the rider is mounted (Carmichel II et al., 2014). The risk of spooking and accidents has been shown to increase when the handler is mounted and as speed increases (Hawson et al., 2010). For research to provide insight into how a horse behaves during use in the industry, research should mimic how horses are routinely handled. When there is a human present, the behavior of the horse has been shown to change (Munsters et al., 2012; Vissers et al., 2002). Human presence can change the behavior of the horse and it may change how the horse reacts. Research conducted without a handler, or rider may be different outcomes and results. This could be dangerous to handlers. Handlers may be expecting a horse to react in a specific way because of something found with a learning or novel object test without a handler. However, due to the handler's presence the reaction could be different and could be potentially dangerous.

When taking into consideration the risk of accidents as well as the effect of the human-horse relationship, the human-horse relationship should be considered when conducting research.

Conclusion

More importance should be given to the impact of the handler on the horse and how this changes horse behavior in certain situations. This will allow research to more mirror the industry and better assist with improvement in understanding equine perception and training methods. Horses have been known to react to subtle changes in their environment, even so subtle as a rotated object. This phenomena has been evaluated allowing the horse to roam free and using latency to eat and concluding that horses were able to recognize the rotated object (Hanggi 2010). None-the-less, anecdotally, handlers still see horses react and spook at subtle changes in their environment. Further research needs to be done to evaluate how different methods of handling and training affect the horses' reaction to changes in their environment.

Chapter 2 will look into horses reaction to a previously familiar rotated complex object after habituation. Chapter 3 is a continuation of research in Chapter 2, evaluating how horses reactions lessen overtime when exposed to a rotated orientation of a previously familiar complex object.

CHAPTER 2: EVALUATING THE RECOGNITION OF A LARGE ROTATED OBJECT IN DOMESTIC HORSES (EQUUS CABALLUS)

Introduction

Research surrounding equine visual perception is very limited. There is seven times more research done on rat cognition than horse cognition (Cooper, 2007). Many have noted the value in more research on equine perception. By learning more, care, training and management practices can improve to better benefit the overall welfare of horse (Heitor and Vicente 2007, Goodwin et al., 2009, Brubaker and Udell, 2016). Anecdotally, trainers and riders often find that their horses may startle at things that were previously familiar. These instances can often be witnessed when a garage door is open when it is usually closed or a chair facing a new direction. This is not because the horse lacks the ability to remember things. Horses have exceptional memories and have been shown to remember how to solve mazes after an interval of a week (Mariner and Alexander, 1994) or even as long as a month after (Wolff and Hausberger, 1996). Additionally, discrimination learning between objects are retained as long as seven years (Hanggi and Ingersoll, 2009). Still, in certain situations, a horse may react suddenly to a familiar object it has previously observed. These reactions can cause dangerous accidents, especially if the rider or handler is not expecting it. It is possible that the orientation of an object may play a role in the horse's ability to recognize it as something familiar or that something might be different. Even though the horse has already been exposed and habituated to the object in a different position, it still may react to a rotated position as though it has never seen it before.

Habituation is described as a lessened reaction or reduced avoidance to a once novel stimulus over repeated exposure (Cooper, 1998). Habituation has been shown to reduce fearful reactions as well as physiological signs of fear by repeated exposure to novel objects (Leiner and Fendt 2011). Habituation enables horses to filter out non-threatening stimuli so they can focus on what the horse perceives as important or life-threatening (Hanggi 2005). For example, a new feeder in a horse's pen may be initially perceived as dangerous, but with habituation, the horse will learn to use the new feeder.

Exposure to novel objects has been shown to induce behaviors associated with fear in horses, as well as other livestock animals, such as head/neck raising and avoidance movement (horses: Leiner and Fendt 2011, Christensen et al 2005, Visser et al 2002; cattle: Grandin 1997; pigs: Dalmau 2009). The horse's fearful behavior has an adaptive value. It alerts the horse to novel things in its environment and can trigger a flight response if the object is perceived as dangerous. Since they are prey animals, horses need to be aware of potential danger at all times. There may also be fear-inducing situations in the environment of domestic horses such as novel objects in either familiar or unfamiliar locations. These situations may be a wheelbarrow at a horse show, a trash can on the road, a downed tree on the local trail path or a new banner in the arena the horses work in every day. These environmentally-induced fear-based responses can lead to dangerous outcomes, particularly for at-risk populations such as youth riders or riders with disabilities.

Leiner and Fendt evaluated behavioral signs that are shown when a horse is exposed to a novel object (2011). The behavioral signs recorded by Leiner and Fendt (2011) were "ears pointing towards the object plus focusing, elongation of the upper lip, tense neck muscles (elevated neck), snorting (short powerful exhalation from nostrils), snuffling, avoidance behavior leaning backwards, avoidance behavior with evasive movements (steps to the side), avoidance behavior with evasive movements (steps) back and flight behavior (jumping away in a sudden movement, typically followed by trotting/galloping)". These behavioral signs were also validated with heart rate monitors. The authors noted that heart rate went up at sight of the object, just before any avoidance behavior (Leiner and Fendt, 2011).

Horses may react to sudden changes in their environment, but little is known about their perception of and ability to categorize changes in familiar objects. Their ability to recognize sudden changes serves a purpose to horses as they are prey animals, but their reactions have potential for dangerous situations. The purpose of this study was to use these behavioral signs that are associated with fear (Leiner and Fendt 2011) to evaluate if horses will react to a familiar large object when it is rotated ninety degrees.

Materials and Methods

This study was approved by the Colorado State University Animal Care and Use Committee. The experiment was non-invasive and used procedures normally used to train horses.

Animals

The sample population consisted of twenty-nine American Quarter Horse 2 and 3-year-old fillies and gelded colts in a university horse training program. These previously untrained young horses had less than 4 months of training at the time of this study. This training included haltering, leading, lunging, and acclimation to being groomed and handled. For the purpose of this study, the young horses needed to be safe and manageable for the handler while leading. Consequently, five of the twenty-nine young horses were pulled from the study due to their lack of safe handleability. Four additional horses were pulled on day 3 due to their inability to safely acclimate to the testing environment. Twenty horses continued in the study (n=20). They were housed at the Colorado State University Equine Teaching and Research Center (CSU ETRC) in outdoor pens with ad libitum water and access to shelter. Each pen was fed a mix of grass and alfalfa hay.

Test environment

The test environment was an alley in an indoor horse barn in front of empty stalls with doors closed. There was an opening in the alley with room for observers and the novel object (Fig. 1). Horses came in through the entrance, walked down the alleyway, past the novel object and left through the exit. (Fig. 1). There were three observers positioned in the test environment and they were still for the entire study. Two GoPro Hero 5 video cameras were placed in the test environment as a secondary observation method.

Figure 1.

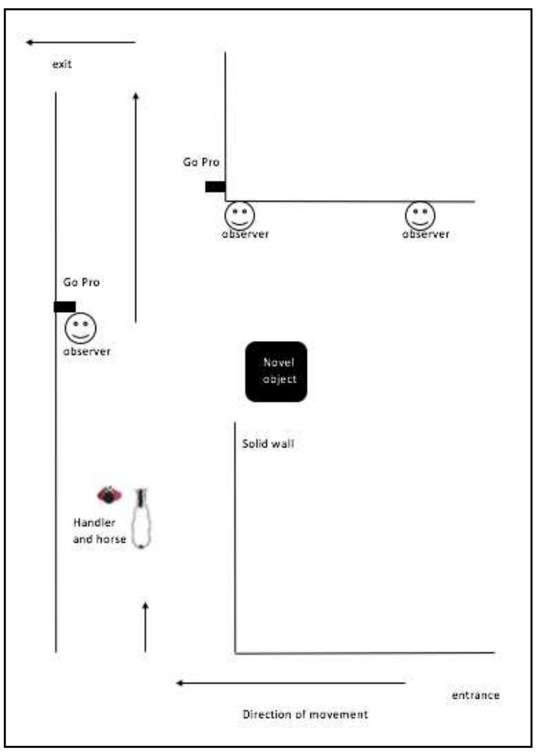

Figure 1. The test area showing observers (☺), GoPros (▬), direction of movement, and where the novel object is placed during days 4-6 (habituation to the novel object) and day 7 (rotation day).

Test Object

The novel object was a children's plastic playset (Little Tikes Hide and Seek Climber and Swing - Brown and Tan) (Fig. 2). The object was 134.62 l x 132.08 w x 104.14 h cm. This object was used because, in both orientations, its outer dimensions are similar. The playset has a different shape when rotated. Rope halters with 2 m lead ropes were used to lead the young horses past the playset.

Behavioral Signs

Observers recorded ten different behavioral signs during each pass on each day: ears focused on the object, nostril flares, neck raising, defecation, snorting, snuffling, avoid lean, avoid side, avoid back, and avoid flight (Table 1).

Table 1. Behavioral Signs: Behavioral signs and definitions used for behavioral analysis (adapted from Leiner and Fendt 2011).

Definitions of Behavioral Signs	
Behavioral Sign	**Definition**
ears focused on the object	ears are alert and focused on the novel object
nostril flares	nostrils flaring more than just normal breathing (nose elongation)
neck raising	neck raised above normal headset and/or neck muscles tense
defecation	defecation
snorting	short powerful exhale
snuffling	long sigh-like exhale
avoid leaning	avoiding the object by leaning away, without feet moving
avoid side	avoiding the object by evasive steps to the side
avoid back	avoiding the object by evasive steps backwards
avoid flight	avoiding the object by jumping away in a sudden movement

Testing procedure

Each horse was led at a walk through the test area by the same handler for the entire study. The handler wore the same clothes each day (black overalls, a jacket, a hat and black boots). The handler was instructed to walk the horse with a lead rope through the alley, and move with the horse, only stopping or turning when the horse stopped or turned towards the object. If the horse stopped, the handler waited 5 seconds before gently encouraging the horse forward. To facilitate habituation, horses were allowed to investigate the novel object for a period of 5 seconds.

Table 2. Testing Procedure: Outline of testing procedure providing details of what each group will be doing each day.

Testing Procedure			
Days 1-3	Habituation to test area (Without novel object)	Control and Rotated groups	
		5 passes each day 15 total passes Test area (Fig. 1) without novel object	
Days 4-6	Habituation to novel object	Control and Rotated groups	
		5 passes each day 15 total passes Test area (Fig. 1) with novel object in original position 	
Day 7	Rotation day	Control group	Rotated group
		1 pass Test area (Fig. 1) with object in original position 	1 pass Test area (Fig. 1) with object in rotated position 

Habituation to test area

On day 1-3 of the study (habituation to the test environment) (Table 2), the horses were led through the test area five times without the novel object to habituate the horses to the test environment. On the fifth pass of each day, if the horse was still showing more than three behavioral signs, it was given one additional pass to encourage habituation. This was only necessary on the first day of habituation, where two horses showed a reduction in fear-related behavioral signs after the additional pass. Horses that displayed more than one behavioral sign on the last pass of the third day of habituation were considered not habituated to the test area and were removed from the study. Four horses were ultimately removed on day 3 due to lack of general acclimation to the test area. After the third day of habituation twenty horses remained in the study.

Habituation to novel object

On day 4, the novel object was placed in the test area and behavioral signs were observed and recorded. Days 4-6 of the study (habituation to the novel object) consisted of the same procedure for the first three days with the novel object in its original position (Table 2). None of the horses needed an extra pass by novel object to become habituated. All horses showed one or less behavioral signs on the last pass of the last day and were considered habituated to the novel object.

Rotation Day

The horses were randomized into a control group (seven fillies and three colts) and a rotated group (six mares and four geldings). On day 7 (rotation day), the control group had one single pass (pass 16) through the test area, with the novel object in the original position (Fig. 2). The rotated group was led through the test environment for one single pass (pass 16) with the novel object rotated 90 degrees clockwise (Fig. 2).

Behavioral Analysis

The behavior signs observed are described in Table 1. Snuffling, defecation, avoid by moving feet back and avoid by flight were later excluded from analysis due to the infrequency of the behaviors.

Stopping and/or hesitating before approaching the object was later analyzed using the video from the GoPro Hero 5 footage and categorized into either stopping or not.

Statistics

The changes in number of behavioral signs shown in each horse on pass 1 (habituation to novel object) versus pass 16 (object in rotation) were analyzed with an unpaired two-sample Wilcoxon test using R. The change in stopping behaviors observed in each horse on pass 1 and pass 16 were analyzed with a Fisher's exact test using R.

Results

The behavioral signs observed during the study were analyzed. Later, stopping behavior was observed and recorded from the videos taken for each horse.

Behavioral Signs

Results of the changes in the number of behavioral signs observed between pass 1 (habituation to novel object) to pass 16 of the control (no change in object) and the rotated group (object in rotation) showed significant differences ($W = 9.5$, $p = 0.001572$, $p < 0.05$). The control group had a major reduction in the average changes in number of behaviors from the first pass to the last pass with no change in the object. The average number of behavioral changes observed in the rotated group illustrated that they reacted similarly to the object on the first pass as they did on the last pass with the object in rotation. Figure 2 shows the percentage of horses showing behavioral signs in the control group and the rotated group comparing first pass by the novel object and the last pass in the rotated position for the rotated group and no change for the control group.

Figure 2

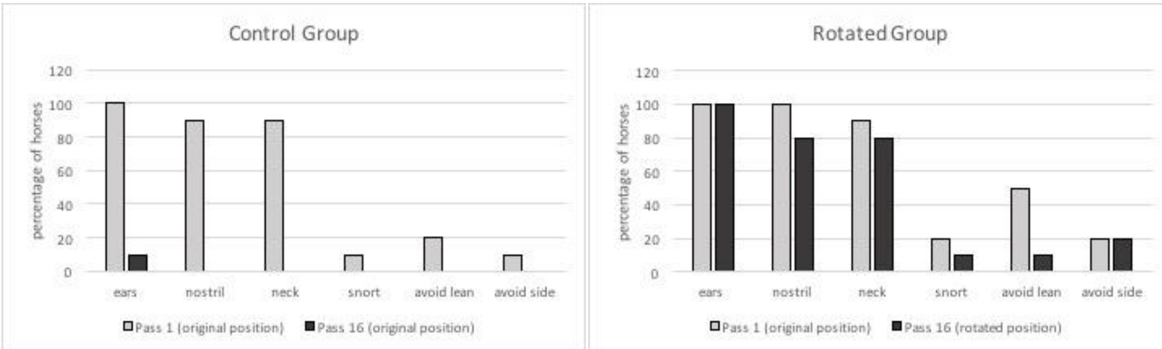

Figure 2. Graph of Behavioral Signs: The number of horses that showed behavioral signs on pass 1 by the novel object (original position) and pass 16 by the object for the control group (original position) and the rotated group (rotated position).

Table 3. Behavioral Signs observed: The number of horses that showed behavioral signs during pass 1 by the novel object and pass 16 by the object for the control group and the rotated group.

Number of horses showing behavioral signs (control group)						
	ears	**nostril**	**neck**	**snort**	**avoid lean**	**avoid side**
Pass 1 (original position)	10	9	9	1	2	1
Pass 16 (original position)	1	0	0	0	0	0

Number of horses showing behavioral signs (rotated group)						
	ears	**nostril**	**neck**	**snort**	**avoid lean**	**avoid side**
Pass 1 (original position)	10	10	9	2	5	2
Pass 16 (rotated position)	10	8	8	1	1	2

Stopping Behavior

There was such a reduction of stopping behaviors in the rotated group on pass 16., that there was no significant difference when compared to the control group ($p = 1$, $p < 0.05$) (Figure 4). This shows that despite the behavioral reactions shown earlier, they still show a reduction in hesitation to the object in rotation. Over half of the horses that stopped at the novel object on the first pass, did not stop on the last pass with the object in rotation.

Table 4. Stopping behavior: Stopping behavior for pass 1 by the novel object (original position) and pass 16 by the object for the control group (original position) and the rotated group (rotated position).

Stopping Behavior				
	Control group		**Rotated group**	
	pass 1	pass 16	pass 1	pass 16
stopped	5	0	9	4
did not stop	5	10	1	6

Discussion

All horses in the rotated group (n=10) reacted to the rotated object. These reactions varied in intensity but were just as significant as the original reactions on pass 1 (when the object was novel). The behavioral reactions suggest the young horses either did not recognize the object after it was rotated, or perhaps responding to a sudden change in the object. Furthermore, over half of the young horses were more willing to approach the rotated object on the last pass, versus when the object was novel on pass 1. The lack of stopping behavior indicates that they may have recognized something familiar about the rotated object. Future studies are needed to determine whether reactions came from truly a non-recognition of the rotated object or from a sensitivity to a sudden change in the environment. These findings could have major implications for better practices in ensuring safety in future horse training and management.

Figure 3.

Figure 3. Behavioral signs by individual horse (rotated group): Number of behavior signs shown on each pass 1-16 with the object rotated on pass 16.

The results of this study confirmed that horses will react to sudden changes in an object's orientation. This is seen in the horses' behavioral reactions on pass 16 in the rotated group (Fig. 3). It was observed that all of the horses in the rotated group displayed varying reactions to the rotated object, including ears focused on the object, nostril flares and neck raising. It is important to note for safety that horses may not react the same to all orientations or environments. In this study, the only change in the environment from pass 15 to pass 16 was the object. This is vital information for training or riding horses in new or changing environments. A horse may be ridden every day in the same arena, but one day he startles without an obvious reason. Objects viewed in different orientations may often be visible during trail rides. If a horse had only seen bikes from the side view, he may perceive a bike from the front view as a reason to react. These situations may be dangerous for the rider especially if the horse is being ridden in a familiar place and the rider is not expecting any problems. This information is especially important in Equine Assisted Activities and Therapies (E.A.A.T). Large objects such as wheelchairs, mailboxes, basketball hoops, and even playsets are often used with patients when they are riding or working on the ground with the horse. Knowing how a horse may react to different orientations of a large object is very helpful when training horses and conducting E.A.A.T. sessions. Additionally, many E.A.A.T. clients use wheelchairs making this information important for safety. Being aware of this and training accordingly can help prevent the horse from being startled which may put a patient or other handlers at risk.

It is also important to remember that in this study, the horses were walked slowly past the novel object by a handler. If the horses had been ridden or moving faster past the rotated playset, it is likely that their reactions would have been larger and possibly more dangerous. Hawson et al. (2010) found a lessened risk of horse related human-injuries at slower gaits. In another study, horses with minimal training or less exposure to being handled may have a larger or more dangerous reaction to novelty (Lansade et al., 2004). For safety reasons, we deliberately avoided faster speeds, unhandled or overly reactive horses.

Recognition of rotated object has been studied before in horses. Hanggi (2010) found that categorization of six small plastic children's toys (toy wheelbarrow, lawn mower, tractor, truck, dinosaur and lizard) was possible with food reward (positive reinforcement) in four horses. The horses were able to choose the "correct" object when it was viewed in a rotated position. The main differences between this study and Hanggi's findings was that positive reinforcement in the form of a food reward was used to reward recognition of the object. The objects were also much smaller than the children's playset used in the present study. It has been shown that larger objects appear as more important to the horse (Uller and Lewis, 2009). This study affirms that horses may react to sudden changes in familiar objects or environments.

Conclusion

Horses react to a rotated object even after being habituated to its original orientation. Sudden changes in a horse's environment, even so subtle as a rotated object, can cause them to react. While they may still startle at the rotated orientation, their willingness to investigate does indicate that they possibly recognize something familiar about the rotated object. While the horses may have some sort of recognition of a rotated object, their reactions still show that they notice some difference in the object.

CHAPTER 3: EVALUATING THE REACTION TO A COMPLEX ROTATED OBJECT IN DOMESTIC HORSES (EQUUS CABALLUS)

Introduction

Horses are prey animals and they exhibit behaviors that help them adapt and survive in their natural environment. Fear reactions to perceived danger are essential for their survival. These reactions are often referred to as a spooking and have the potential to be dangerous to the horse, handler and rider. 'Spooks' are avoidance reactions that include suddenly moving away or running away from the perceived danger. To prevent horses or other prey species from having constant flight reactions, they must be able to filter out unimportant stimuli (Hanggi, 2005). Horses can learn what is safe and what is perceived as dangerous. When presented with novel objects in their environment, horses will slowly become accustomed to them through repeated exposure. This is known as habituation (Cooper 1998). Horses use habituation and many other different learning tools to process and survive in their natural environment. Despite this adaptation, domesticated horses have a tendency to sometimes spook at objects they have previously seen and been habituated to. However, there is not much research on this phenomena. Additionally, there is little research on equine visual perception and cognition in general, compared to other species (Nichol, 2002).

Anecdotally, many people in the equine industry have observed that horses will react, sometimes violently, to a familiar object when they see it in a different orientation. This reaction was thought to be due to horses not recognizing the object when it is in a new position. This concept was evaluated by Hanggi (2010) using discrimination and food reinforcement, or positive reinforcement, to evaluate whether a horse could identify an object that had been rotated. The authors found that horses were able to discriminate between several different orientations of the object after learning through positive reinforcement with food (Hanggi 2010). This research is helpful to evaluate how horses learn when using positive reinforcement. In various studies with horses and other species, the animals were unrestrained and were free to either approach or avoid the novel object (Christensen et al., 2008; Safryghin et al., 2019). However, when there is a human handler present, the behavior of the horse has been shown to change (Munsters et al., 2012; Vissers et al., 2002). Human handlers or riders can change the behavior of the horse and change how the horse reacts. When doing research without a handler, there may be different outcomes and different behaviors.

The human-horse relationship has been shown to have an impact on how the horse reacts to stimuli. Borstel et al (2011) found that when comparing reactivity in temperament tests of horses free-running, being led and being ridden, horses being led and ridden reacted differently than horses free-running. Additionally, A horse's sudden flight reaction can be dangerous and can cause accidents that risk the safety of the horse and human (Angoules et al., 2018). Injuries and accidents in the horse industry are very prevalent. Spooking can be a common cause of accidents (Camargo et al., 2018). The risk of spooking and accidents has been shown to increase when the handler is mounted and as speed increases (Hawson et al., 2010). This risk of handling and riding horses is acknowledged by the equine community due to the unpredictable nature of the horse. (Thompson et al., 2015). While this risk is acknowledged, there should be more efforts to help handlers understand the horse and be better prepared to avoid accidents. Horses' behavior changes when a handler is involved. Additional research may provide insight into how horses might behave when being led by a handler or ridden towards a novel object.

Changes in a horse's environment, as subtle as a rotated object, may cause a horse to spook and cause injury to the horse or person, when the horse is being handled. Horses' reaction to a rotated orientation of a familiar object and reduction in reaction over time will be similar to their original exposure. The purpose of this study was to evaluate how horses being led and habituated to a previously familiar complex object would react after it was rotated ninety degrees.

Materials and Methods

This study was approved by the Colorado State University Animal Care and Use Committee. The experiment was observational and used procedures normally used to handle horses.

Animals

The sample population consisted of twenty-two American Quarter Horse 2 and 3-year-old horses (15 fillies and 7 gelded colts) in a university horse training program. The horses had 4 months of handling training at the time of this study, all trained at the same place. The horses were taught using low-stress methods of pressure and release training to halter, lead, lunge, and acclimate to being groomed and handled. Of the twenty-two horses, one posed a safety concern for the research handlers by its continued attempt to pull away and was removed from the study. Another horse was removed from the study on day 4 for soundness issues. Twenty horses continued through the entire study (n = 20). All horses were housed at the Colorado State University Equine Teaching and Research Center (CSU ETRC) in outdoor pens with ad libitum water and access to shelter. Horses were fed a mix of grass and alfalfa hay once per day on a feed bunk.

Test environment

The test environment was an alley (4.57 meters wide) in an indoor horse barn in front of empty stalls with the doors closed. The barn had concrete flooring and illuminating lights above the alleyway. The horses were led in through the entrance, walked down the alleyway, past the novel object and led out of the test area through the exit (Fig. 4). Two GoPro Hero 5 video cameras were placed in the test environment for later observation of behavioral signs.

Figure 4. Test Area

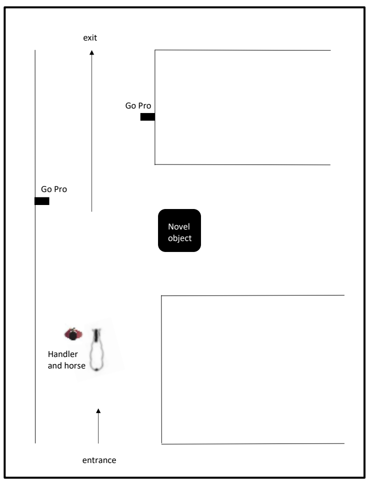

Figure 4. Test Area: The test area consists of GoPros (▬) and the novel object placed during days 4-6 (habituation to the novel object) and days 7-9 (rotation days).

Test Object

The novel object was a children's plastic playset (Little Tikes Hide and Seek Climber and Swing - Brown and Tan) (Fig. 2). The object was 134.62 l x 132.08 w x 104.14 h cm. This object was used because, in both orientations, its outer dimensions are similar. The playset has a different shape when rotated ninety degrees. Rope halters with 2 m lead ropes were used to lead the young horses past the playset.

Testing procedure

Two handlers were used. They both led the horses at a slow walk. Each handler had an equal number of horses randomly assigned from both the control and rotated group. Each horse was led at a walk through the test area by the same handler for the entire study. The handlers wore the same clothes every day (black overalls, jacket, hat and black boots). The handlers were instructed to walk the horse with a lead rope through the center of the alley (1 m away from the object), and move with the horse, only stopping or turning when the horse stopped or turned towards the object. If the horse stopped, the handler waited 3 seconds before gently encouraging the horse forward by walking forward and slightly pulling on the lead rope. To facilitate habituation, if a horse stopped when it was either approaching or passing the novel object, it was allowed to stop for a period of 3 seconds. If the horse did not stop, the handler continued to lead it past the novel object.

Table 5. Testing Procedure: Outline of testing procedure to provide details of the Control and Rotated group procedures.

<table>
<tr><td colspan="4">Testing Procedure</td></tr>
<tr><td rowspan="2">Days 1-3</td><td rowspan="2">Habituation to test area (novel object absent)</td><td colspan="2">Control and Rotated groups</td></tr>
<tr><td colspan="2">5 passes each day
15 total passes
Test area (Fig. 1) without novel object</td></tr>
<tr><td rowspan="2">Days 4-6</td><td rowspan="2">Habituation to the novel object</td><td colspan="2">Control and Rotated groups</td></tr>
<tr><td colspan="2">5 passes each day
15 total passes
Test area (Fig. 1) with novel object in original position
</td></tr>
<tr><td>Days 7-9</td><td>Rotation days</td><td>Control group</td><td>Rotated group</td></tr>
<tr><td></td><td></td><td>5 passes each day
Test area (Fig. 1) with object in original position
</td><td>5 passes each day
Test area (Fig. 1) with object in rotated position
</td></tr>
</table>

Habituation to test area, novel object absent

On day 1-3 of the study (habituation to the test environment) (Table 5), the horses were led through the test area five times each day without the novel object to habituate the horses to the test environment.

Habituation to the novel object

On day 4, the novel object was placed in the test area in the original position. Days 4-6 of the study (habituation to the novel object) consisted of the same procedure for the first three days with the novel object in its original position (Table 5). Each horse passed the original position of the object fifteen times over three days.

Rotation Days

The horses were randomized into a control group and a rotated group. On days 7-9 (rotation days), the control group had five passes each day through the test area, with the novel object in the original position (Table 5). The control group passed the original position of the object fifteen times over the three days. The rotated group was led through the test environment for five passes each day with the novel object rotated 90 degrees clockwise (Table 5). The rotated group passed the rotated position of the object fifteen times over the three days.

Behavioral Signs and Reaction Scale

Behavioral analysis of the videos was performed after completion of the study. One observer recorded eight different behavioral signs during each pass on each day. The behavioral signs recorded were ears focused on the object, nostril flares, neck raising, snorting, avoid stop, avoid side, avoid back, and avoid flight (Table 6).

Table 6. Behavioral Signs: Behavioral signs and definitions used for behavioral analysis (adapted from Leiner and Fendt 2011, with adjustments).

Definitions of Behavioral Signs	
Behavioral Sign	Definition
ears focused on the object	ears are alert and focused on the novel object
nostril flares	nostrils flaring more than just normal breathing (nose elongation)
neck raising	neck raised above normal headset and/or neck muscles tense
snorting	short powerful exhale (McDonnell, 2003)
avoid stop	avoiding the object by stopping, feet stop moving
avoid side	avoiding the object by evasive steps to the side, away from the object
avoid back	avoiding the object by evasive steps backwards, backing up
avoid flight	avoiding the object by jumping away in a sudden movement, feet moving above a walk

A reaction scale was created from the behaviors observed on a scale from 0-3 (Table 7). This reaction scale was adapted from Christensen et al. (2008) to better evaluate reactivity based on behaviors observed in this study.

Table 7. Reaction Scale

Score 0-3	Behavioral Signs Observed
0	No behavioral signs observed
1	Ears focused, nostril flares, and/or neck raising
2	Snorting and/or avoid stop
3	Avoid side, avoid back, avoid flight

Statistics

The difference in the reaction score per individual horse shown on the first pass by the novel object to the first pass by the rotated object was analyzed with a two-sample t test (R). This test was done for each pass 1-15 comparing the corresponding passes from the original position to the rotated position.

Results

The control and rotated group showed significant differences between the change in reaction score from the first pass by the novel object to the first pass on the Rotation Days (T Test p-value = 0.0014)(Fig. 5). Horses that reacted to the novel object in the Rotated Group, reacted similarly on the first pass by the rotated position of the object as they did on the initial pass by the novel object.

Figure 5.

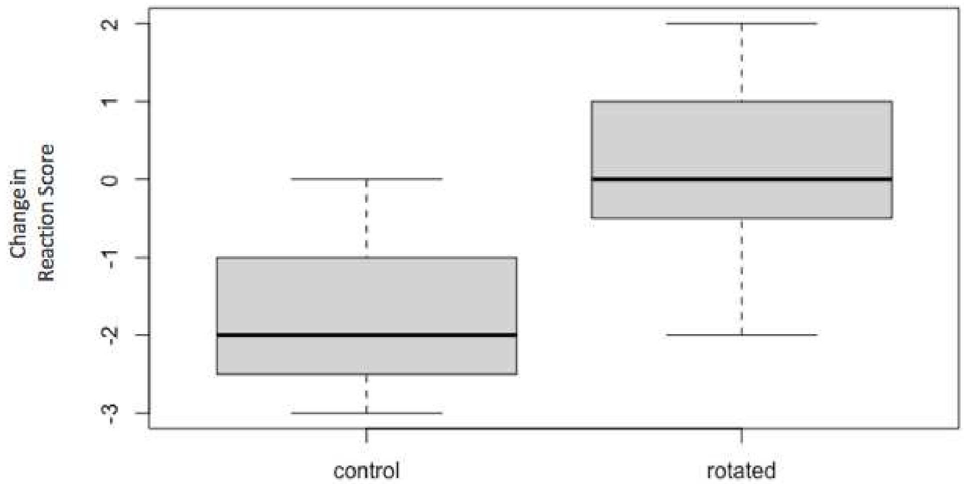

Figure 5. Boxplot of differences in reaction score for Pass 1 by the novel object to Pass 1 by the rotated object.

Figure 6.

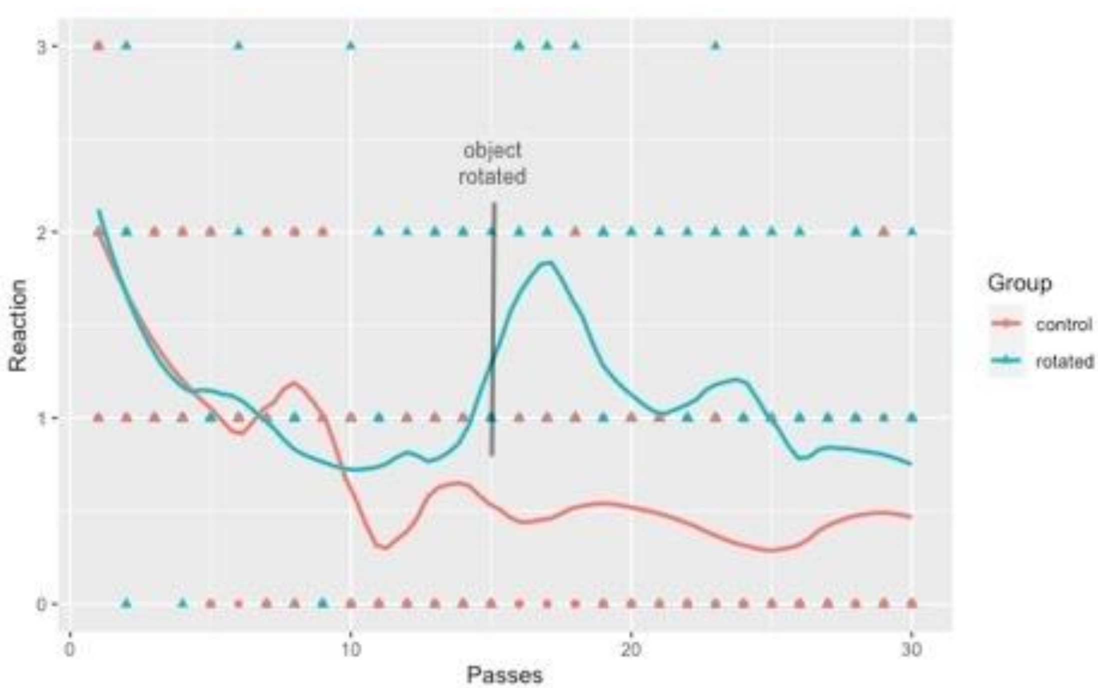

Figure 6. Graph of Reaction Scores from pass 1-30 for the control and rotated groups.

Passes 1-4 after rotation in the Rotated group showed a significant difference between the two groups change in reaction ($P<0.05$). After pass 4 by the rotated object, there was little significant difference between the rotated and control groups (P>0.05). As noted in Table 8, some later passes also showed significant differences in the change in reaction between the two groups (Passes 1-4, 8, 9, 12: P>0.05). Figures 5 and 6 show the significance in the change in reactions for the rotated group when the horses were exposed to the rotated object.

Table 8. Values for Differences in reaction score for corresponding passes 1-15 by the novel object to rotated object.

	Control			Rotated			
Pass #	Mean	Min.	Max.	Mean	Min.	Max.	p-value
1	-1.75	-3	0	0.083	-2	2	0.0014
2	-0.875	-2	0	0.25	-1	2	0.0098
3	-0.875	-2	0	0.167	-1	1	0.0039
4	-1	-2	0	0.333	-1	1	0.0013
5	-0.375	-2	1	-0.083	-2	0	0.312
6	-0.375	-1	0	-0.25	-1	1	0.719
7	-0.5	-2	0	0.167	-1	1	0.0616
8	-1	-2	0	0.333	-1	2	0.0046
9	-0.875	-2	0	0.583	-2	2	0.0018
10	-0.125	-1	1	0.167	-1	2	0.5369
11	0	0	0	0.167	-2	2	0.6556
12	0.125	-1	1	-0.333	-1	0	0.0098
13	-0.125	-1	1	0.333	-2	1	0.226
14	-0.25	-1	2	0.083	-2	2	0.554
15	0	-1	1	-0.167	-2	2	0.7

Discussion

When a previously familiar complex novel object is rotated, the rotated object may cause reactions similar to the initial exposure to the novel object. This confirms what handlers and riders have seen anecdotally. Horses may spook at objects that were familiar, but may have shifted slightly making them look different. Understanding the horses reaction to a rotated object is important for the safety of riders and handlers. If handlers expect horses not to react to subtle changes in a familiar environment, they are less prepared for a horse spooking which could lead to an accident. If handlers are aware of potential reactions to changes of familiar objects, they can be better prepared to avoid an accident.

As shown in Figure 6, there was a steady decline in the horses' reactions with each successive pass by the rotated object. Reactions declined overtime in a similar manner as seen in the decline of reactions to the initial exposure to the novel object (pre-rotation, passes 1-15). Table 8 shows the decrease in the horses' reaction to the object with each succesive pass, for the first four passes. After pass 4, the changes in reactions between the rotated and non-rotated groups seem to be less consistent. This inconsistency in changes in reactions between the two groups shows the unpredictable nature of the horse. Even subtle changes to a familiar object can cause horses to react again These subtle changes can cause the horse to need more exposure until they are habituated or until no reactions are shown again. Additionally, there were a few random passes where one horse in the group showed a greater reaction. These outliers are an example of the unpredictable nature of the horse and individual differences in horse behavior. Handlers need to be aware of this for safety of themselves and the horses.

This study shows that despite previous research (Hangii, 2010), horses may not recognize different orientations of previously familiar objects, when being led by a handler. While assumptions cannot be made about the horse's recognition of the rotated object from the present study, there is an obvious reaction to the rotated object. This reaction is important to note and important for anyone handling horses to be aware of. Humans can have an impact on how the horse reacts and behaves (Visser et al., 2002). The present study shows that the presence of a handler leading the horse could change the result of a study, as compared to a study conducted without a handler. There may be a difference between a voluntary approach, as compared to being led by a handler. The present study did not use food or positive reinforcement when evaluating recognition or reactivity, as compared to Hanggi (2010). Using food as a reinforcement in training is similar to using latency to eat in research. It is important to note, that most trainers do not use food as reinforcement in their training. Training methodologies are worth further exploration when researching equine perception of novel objects.

The purpose of this study was to evaluate how horses being led and habituated to a previously familiar complex object would react after it was rotated ninety degrees. This study showed that horses' reaction to a rotated orientation of a familiar complex object was similar to its reaction when it first saw it. Additionally, their reduction in reaction at the rotated orientation over time will be similar to their reduction in reaction at the original exposure.

Conclusion

Horses may have a greater reaction to new orientations of previously familiar objects. This may cause accidents that lead to injury of the horse or handler. If handlers and riders can be prepared for how a horse may react, they may be able to help reduce risk. Additionally, while horses show a steady decrease in reactions to novel objects and novel orientations of familiar objects, there is the possibility for changes in their reaction during habituation. Having awareness of the unpredictability of the horse has potential to help reduce risk in the horse industry, by better preparing handlers and riders. Further research needs to be conducted to evaluate how different methods of handling and training affect the horses' reaction to changes in their environment.

Chapter 2 shows that horse would react to subtle changes in their environment when being led by a handler. Chapter 3 further evaluated how horses would react to subtle changes in their environment and habituate. This research shows how a handler can change the behaviors of horses and outcomes of research when the horse is being led.

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