Memory Recall in Horses Tranquilized with Acepromazine Maleate

by

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Abstract

Acepromazine maleate (Ace) is a common tranquilizer employed by horse handlers as a "training aid" because it allows handlers more control of fractious horses in training situations. Tranquilizing horses with Ace is effective at increasing tractability in horses, but little research exists on its effect on learning ability and the ability to recall learning at later dates. Thirty-five mature horses were assigned randomly to tranquilized (n = 18) and non-tranquilized control (n = 17) groups and used to determine if recall abilities differed between tranquilized and non-tranquilized horses. Horses were trained for three consecutive days to lever press in a training stall containing a single lever for a food reward with a buzzer as a secondary reinforcer. Horses were trained to a criterion of 30 independent lever presses for each training day (90 total presses). On the fourth day, each horse was administered either 0.088 mg/kg IM of Ace or a saline control, according to treatment, and allowed to stand for 15 min while the tranquilizer took effect. Then the horse was moved into a second stall containing a white lever and a black lever. Each horse was assigned randomly a correct lever color and was trained to criterion of 30 independent lever presses on the assigned lever on that day. Fourteen days after the training date for the lever color discrimination, horses were returned to the two-lever stall and allowed to lever press. Each lever press was recorded as either correct (pressing the originally assigned lever color), which was rewarded with food and the sound reinforcer, or incorrect, which received no food reward or sound reinforcer. Number of correct lever

presses and time (s) for the horse to complete 30 correct lever presses were recorded, and data were analyzed using a t-test. No significant difference was detected in number of correct lever presses between control (29.5 ± 0.3) and tranquilized (28.5 ± 0.3) groups. Similarly, no significant differences in time to complete 30 correct responses (control = 643.9 ± 54.8 s; tranquilized = 583.7 ± 53.2 s) were detected. Six months after the initial two-week testing, 26 of the original horses were retested in the same test stall to determine long-term memory recall ability. Procedures were the same as in the 14-day test except horses were not tranquilized or sham-injected, and they received no reinforcement for the correct response. Horses were allowed to respond for 30 lever presses or until 1 hour passed without a lever press by the horse. Again, no significant difference was detected in percent correct responses between control horses and those previously given Ace during learning sessions (65.5 ± 5.1 and 69.1 ± 5.5, respectively). These results indicate that horses can learn while under the influence of Ace and can retain that learned information to be recalled at a later date. This validates the use of Acepromazine maleate as an appropriate tranquilizer to be used during training exercises of a fractious horse.

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Table of Contents

List of Figures

Introduction

For the past 6,000 years, horses have been used in a variety of ways to suit the needs of humans. They have been used as tools of war, carrying soldiers and pulling carts, as farming power pulling plows, as human and pet food and most recently as a tool for recreational activity. For most of those years, horses were trained with little knowledge of how the horse learned or the best techniques to train them. Learning ability of horses is of vital importance to their usefulness to humans. In the recent past, horses' cognitive abilities have been studied in many areas that include but are not limited to: discrimination learning (McCall, 1989 and Dougherty and Lewis, 1991), concept learning (Sappington and Goldman, 1994), reversal learning (Sappington et al., 1997), avoidance learning (Haag et al., 1980; Rubin et al., 1980), and observational learning (Baer et al.,1983) Although there has been great expansion in knowledge of the learning ability of horses, very little has been uncovered on the effects of pharmaceuticals on those processes.

Owners who have little or no previous experience with large livestock species utilize the majority of horses in the U.S. for recreational use. Therefore, horse owners often are quick to resort to fast and easy solutions, such as tranquilizers, to reduce problem behavior in the horse. Additionally, the size, mobility and unpredictability of the horse tend to create a dangerous situation for owners and handlers attempting to perform mildly aversive training (e.g. loading in a trailer) or management (e.g. veterinary care, clipping) procedures. In these situations, tranquilization often is used to diffuse the reactive nature of the horse and make it more tractable. Previous research has indicated that horses can learn very simple spatial and discriminative tasks while tranquilized. However, training situations generally involve more complex tasks and require recall of the learned task at later date. Therefore utilizing tranquilizers while the horse is being exposed to more complex learning situations is often a controversial subject among horse trainers.

Lever pressing is a common tool employed by researchers as a means of measuring learning ability in many species. The process of teaching the horse to press a lever is relatively easy and the horse adapts to the apparatus without much human interference. Using the lever press and pairing it with a simple visual discrimination task allows the researcher to precisely quantify the learning ability of the horse.

The purpose of the present study was to compare memory recall in tranquilized and non-tranquilized horses on a discrimination task. Results of this study will provide a better understanding of equine learning and memory recall, which may lead to more effective training protocols and horse handling and management practices.

Literature Review

Tranquilizers

Tranquilizers are a commonly used pharmaceutical for treatment of animals that are fractious or anxious. These pharmaceuticals allow more control over the animal and contribute to the safety of the handler and animal. Acepromazine maleate (Ace) is a tranquilizer commonly employed by horse handlers as a training aid. Ace is a common tranquilizer and is easily accessible to the horsemen with veterinary prescription. Ace is a compounded medication with chemistry of 2-acetyl-10- (3-deimethylaminopropyl) phenothiazine hydrogen maleate ($C_{23}H_{26}N_2O_5S$) [Fig 1].

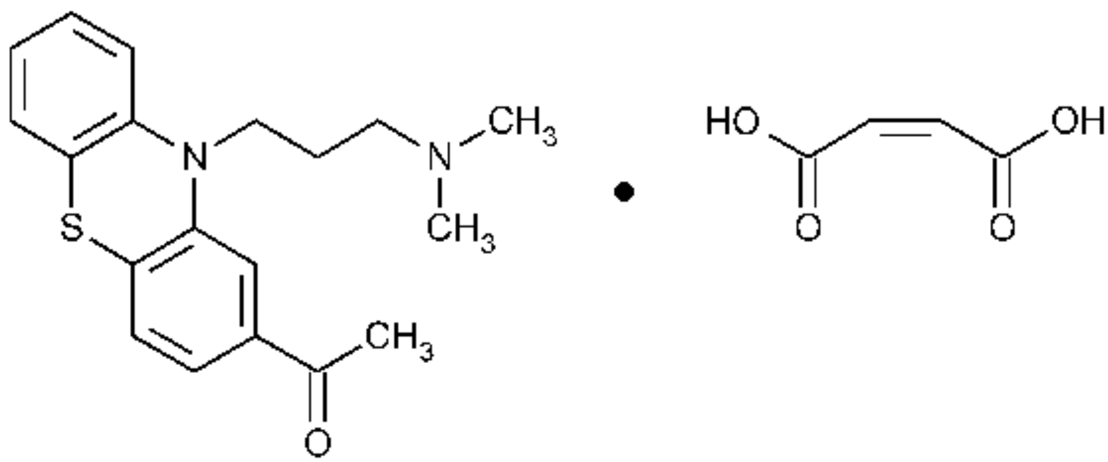

Figure 1. Acepromazine maleate chemical structure

Ace is derived from phenothiazine, which is classified as an antipsychotic and is used to treat schizophrenia or psychosis in humans. Phenothiazine acts as a dopamine receptor antagonist that decreases the effect of dopamine in the central nervous system (brain). Ace has a depressant effect on the central nervous system and, therefore, causes sedation, muscular relaxation and a reduction in spontaneous activity (USDA, 2011). It is classified as a potent neuroleptic agent, a tranquilizer that is most effective when the desired result is a calming effect. As a neuroleptic, Ace acts rapidly, exercising a prompt and marked calming effect. It also lacks a hypnotic effect when administered which is an added benefit in training situations in which cognition is required. Ace has a low order of toxicity that allows it to be useful as a tranquilizer because elevated dosages do not often result in adverse reactions to the compound. The most commonly reported adverse reactions to Ace were mild irritation at administration sites and slight respiratory distress (reverse sneeze). These effects do not have any effect on the desired action of the drug. It should be noted that phenothiazines may potentiate the toxicity of organophosphates and should not be used in conjunction with organophosphorus vermifuges or ectoparasiticides.

Acepromazine maleate is intended for use as a tranquilizer that is effective as a pre-anesthetic agent and lowers the dosage requirement of general anesthetics. It is intended for use in dogs, cats and horses, which are non-food animals. Federal law prohibits the use of this product in animals intended for human consumption (USDA, 2011). Ace can be administered as an oral tablet, an oral paste or as an injectable sterile solution. Sedative action is set within 15 to 20 minutes after injection and 20 to 30 minutes after oral administration (Hashem and Keller, 1993). Effects are maintained for 6 to 12 hours depending on level of sedation desired. The injectable solution can be administered intravenously, intramuscularly, or as a subcutaneous injection. If administered intravenously, it should be done so slowly. Cardiovascular collapse can occur after rapid intravenous injection due to hypotension. Epinephrine should not be used as treatment for ace induced hypotension because it can further depress the blood pressure of the animal. As a general rule, the dosage requirement in mg/lb. of body weight decreases as the weight of the animal increases. Dosages should be individualized depending on the degree of tranquilization desired. Recommended dosages for horses are 0.044-0.088 mg/kg of body weight (ANADA, 2004).

Intravenous injections of Ace are absorbed very quickly and have a half-life of less than 3 minutes versus the half-life of 0.84 hours for oral tablets with slower absorption rates (Marroum, et al., 1994). These data indicate injectable Ace is more effective as an immediate source of tranquilization while oral Ace is more effective with long duration tranquilization. With intravenous injection, peak tranquilization is reached at approximately 10 minutes after administration in comparison to 40 minutes for oral paste and 35 minutes for oral tablet (ANADA, 2004). Hemodynamic effects peak at 100 minutes for intravenous injection and for oral administration (Marroum et al., 1994). When using intravenous injection, the most sensitive hemodynamic effect is the packed call volume. Packed cell volume can decrease up to 20% (Marroum et al., 1994) leading to hypotension in the horses. Systolic blood pressure also deceases after Ace administration and is more pronounced after intravenous injection than after oral administration. With either intravenous or oral administration, heart rate remains unchanged when Ace is metabolized (Leise et al., 2007). Griffith (2006) reported that mean heart rate for un-tranquilized (55.1 BPM) and tranquilized horses (51.3 BPM) showed no statistical difference, which further validates Leise et al. (2007) findings.

Although Ace is administered through the blood with an intravenous injection, there is no noticeable change in the blood pH or in blood gas tensions (Marroum et al., 1994). Penile prolapse is more prominent when Ace is administered intravenously than orally or intramuscularly (Leise et al., 2007). These parameters indicate that the route of intravenous injection may contribute to the adverse effects of acepromazine. It is important to iterate that intravenous injections have to be given slowly because administration rate directly affects hematocrit and blood pressure in the horse, which can contribute to the adverse effects of acepromazine.

Intramuscular administration of acepromazine is the most typical administration route in the horse. Onset of tranquilization occurs approximately 15 minutes after administration when using intramuscular injection. Hematocrit response and decreases in packed cell volume and systolic blood pressure are dose dependent in horses irrespective of administration route (Leise et al., 2007). However, intramuscular injection is a safer option to intravenous injection because there is a lower chance of inducing hypotension in the horse, which can lead to hypothermia, cardiac dysrhythmia and other life-threatening situations. The hypotensive effect of acepromazine is related to both the dose and route of administration. In general, intravenous administration produces a more rapid maximum hypotensive effect than intramuscular injection, and the larger the dose the longer blood pressure remains at low levels (Parry, 2008).

Acepromazine does not produce a complete loss of coordinated motor function or alertness; only partial effects occur when given in low dosages. This feature allows the use of Ace prior to and immediately following exercise of the horse. It has been reported that exercise could increase elimination of basic drugs (Ma, 1990). This statement rings true when dealing with acepromazine in small dosages. When given in multiple, small doses (0.044 mg/kg every 2 hours) and paired with exercise, Ace is eliminated more efficiently than in non-exercised horses, and pharmacodynamic effects are largely diminished (Chou et al., 2002). When administered in single, large dose, Ace remains in the body system longer, and pharmacodynamic effects are more apparent when coupled with exercise (Chou et al., 2002). Therefore, horses subjected to exercise eliminate Ace more efficiently than non-exercised horses, and multiple, small dosages significantly reduce ace resident time in the body (Chou et al., 2002).

Ace also can be used for tranquilization immediately following maximal exercise as an emergency drug. Acepromazine can be administered one minute following exercise, which is beneficial when dealing with post-exercise injury. Because exercise can be detrimental to drug effectiveness, the fact that Ace can be used in a large dosage without a major change in mode of action makes it beneficial as an emergency tranquilizer (Hubbell et al, 2002). In most cases, the tranquilizer will be used as a pre-anesthetic that produces an overall calm demeanor with relaxation and indifference to the surroundings (Hubbell et al., 2002). This combination of effects allows handlers to more safely handle the injured or exhausted horse. It is best to use a muscle relaxing sedative (diazepam, zolazepam, guaifenesin) in conjunction with Ace for the best quality of induction of and continued anesthesia. It is not recommended to use ketamine with Ace because the neuroexcitatory activity of ketamine interferes with the dopamine receptor activity of Ace and causes induction failure, inadequate muscle relaxation and shortened duration of recumbency (Hubbell et al., 2002).

Discrimination Learning in Horses

Discrimination learning is based on the ability to learn to respond differentially to different stimuli (Miyashita et al., 2000). This type of learning is very simple and easy to quantify. Generally, the task of discrimination testing is to achieve the greatest number of correct choices, which can be interpreted as greatest increase in learning.

Discrimination tasks usually are paired with positive reinforcement. Positive reinforcement is defined as the addition of a stimulus or event that results in a change of behavior (McLean and Pratt, 2005). Within positive reinforcement, you can have a primary or secondary reinforcer or both as the presented stimulus that results in a behavior change. Primary reinforcers consist of stimuli that have an evolutionary basis such as food, air, sleep, water and sex. A primary reinforcer does not require a learning element for reinforcement to occur (Rothschild and Gaidis, 1981). A secondary reinforcer is a stimulus that becomes rewarding by being paired with another reinforcing stimulus (Rothschild and Gaidis, 1981). Examples of secondary reinforcers are auditory sounds (clicker or buzzer) or a visual stimuli (smiling or hand gesture). Studies indicate that secondary reinforcers are useful in horse training situations, but that they should be paired periodically with a primary reinforcer to maintain their effectiveness (McCall, 2002). Pairing a primary reinforcer (food) with a secondary reinforcer (buzzer) results in a greater likelihood for learning than if just utilizing a primary reinforcer (McCall et al., 2002).

Early reports of discrimination testing in horses started with a simple task of distinguishing between a feed box covered with cloth and a feed box not covered with cloth (Gardner, 1933). That study concluded that horses can successfully complete a discrimination task and led to further experimentation of discrimination tasks in horses. In a second study, Gardner (1936) reported that horses learned to find food in one of three feed boxes, the one having a black cloth over it. This study showed that all horses learned, and that younger horses learned the signal more readily than older horses but the differences were not great (Gardner, 1936). Also no gender differences in learning were noticed.

After determining that horses are capable of mastering discrimination tests, researchers utilized discrimination tasks to explore differences in learning abilities among different breeds, genders, ages, social dominance status and body condition of horses (McCall, 1989). Flannery (1997) used a discrimination task to demonstrate that horses can learn the concept of sameness, and that they are able to generalize this learning to a novel stimulus presentation situation. Flannery accomplished this using three horses that were shaped to touch individually presented stimuli with their muzzles and then to make responses to two matching cards from an array of three. Before the experiment began, each horse was presented with two similar cards that they were expected to remember for the experiment. The horses' task during the experiment was to ignore the nonmatching cards and to select the cards that were presented before the experimentation began. These results suggest that a relational discrimination test may be useful for assessing horses' learning abilities and the level of training appropriate for individual horses (Griffith, 2006).

Sappington and Goldman (1994) were able to provide insight into concept formation by utilizing discrimination learning in Arabian horses. Their study determined that horses are able to distinguish between complex patterns and that they may also have the ability to form and use concepts in problem solving. This provided insight into how a trainer could apply prior learning experiences of horses as a training technique for a future response. It also suggests that generalization of a horse's experiences could aid in learning a new concept.

Dougherty and Lewis (1991) used stimuli generalization, discrimination learning and peak shift in horses to determine that horses respond in the same way as other organisms used in research facilities. They applied these aspects of stimulus control to lever-pressing behavior and concluded that horses did respond to stimulus shaping and control in the same way as other organisms indicating that horses are good subjects to use for learning experiments.

When using discrimination testing, it is important to know the visual ability of the subject because the test is often based on the subject's ability to distinguish between two visual categories. Macuda and Timney (1999) reported horses perform well in visual discrimination testing at high levels of luminance, which is to be expected. Smith and Goldman (1999) used two-choice color versus gray and achromatic light-dark discrimination tasks to determine color discrimination in horses. Their results indicated that horses have color vision that is at least dichromatic, although some individuals may experience partial color-blindness. It also was determined that horses do not distinguish yellow and green from grey with great accuracy, while they can distinguish blue and red from grey which further supports the dichromatic nature of the horse eye. Geisbauer et al. (2004) reported horses cannot readily distinguish between varying shades of grey, which further supports dichromatic vision in the horse.

While color can be an important part of a visual discrimination test in the horse, height of the stimuli is also an important component. According to Hall et al. (2003) visual discrimination training in horses can be enhanced by placing the stimuli on the ground. Their study suggests that the visual appearance of the ground surface is an important factor in both horse management and training. Their study consisted of using a simple discrimination test with stimuli at ground or nose level to predict which would result in improved learning. While the results indicated that ground stimulus was the better option, it did not indicate that horses were incapable of learning a stimulus placed at nose level. Their results also indicated that presenting a food reward directly beneath a nose-level stimulus could increase learning in the horse.

In discrimination testing, a subject must distinguish between two choices and the experimenter has the option to reward these choices. Miyashita et al. (2000) utilized two different reinforcers (carrots or pellets) to determine if one would better reinforce equine behavior than the other. The researchers randomly assigned one of the food reinforcers to each lever and tested to see if the horse would choose one specific lever/food combination over another lever/food combination. The results indicated that percent outcome of choosing the correct lever (80-90 percent) was not contingent on the lever/food combination. This indicates that there does not need to be a specific type of reinforcer to obtain a response, just that a reinforcer is required. Miyashita et al. (2000) also tested the outcome of using different colored screens in a two-trial discrimination sequence. They concluded that performance on the second trial was affected by the color of the screen of the preceding trial. The horses performed better when the colors of successive trials were the same than when the colors differed between trials (Miyashita et al., 2000). This indicates that when using a discrimination test, it is best to use a set color and to continue using that color throughout the trials for best learning response in horses.

While there is limited pharmaceutical research on equine learning behavior, Griffith (2006) used simple spatial and visual discrimination tests to conclude that horses, while under the influence of acepromazine, had the same learning performance of their counterparts who were not under any pharmacological influence. Griffith (2006) determined that Ace was an appropriate training aid for less skilled horse handlers when performing aversive procedures on fractious horses. This study opened a gateway to follow up research in the field of behavioral pharmacology in horses.

Based on the results of these previous studies mentioned, it can be concluded that discrimination testing is a suitable way to investigate learning ability and memory recall in horses. While these studies explored many aspects of equine discriminative learning ability, they did not investigate the effect of a pharmacological tranquilizer, such as Ace, on complex learning tasks. Because Ace is commonly used in horse management and training situations, it would be beneficial to know its effects on the horse's ability to learn complex tasks and recall that learned behavior at a later date. The objective of this study is to compare memory recall of tranquilized and non-tranquilized horses using a visual discrimination test.

Materials and Methods

Subjects

Thirty-five mature (4 to 24 years) mares and geldings of Quarter Horse, Thoroughbred, and Warmblood breeding were selected for this study. Horses were housed at the Auburn University Horse Center, were utilized in teaching and riding activities at the Horse Center and were accustomed to human contact and handling.

Horses were maintained on predominantly Coastal Bermudagrass (*Cynodon dactylon)* pastures and received a daily concentrate (Southeast Performance Pellets, Cargill Animal Nutrition, Minneapolis, MN) formulated to meet their nutritional requirements. Horses were allowed free access to pasture forage during all non-testing hours. Fresh water was available in the pasture *ad libitum*.

Testing Procedure

Horses were assigned randomly to either the control (0.088 mg/kg of 0.9 % saline intramuscularly; 17 horses) or to the tranquilized group (0.088 mg/kg acepromazine maleate intramuscularly; 18 horses). Horses then were assigned randomly a correct color lever (black or white) that remained their correct response lever throughout the study. Horses were brought into the test area in random pairs and worked consecutively to minimize disturbances caused by separation from the herd, and they were tested during similar times each day to minimize disturbances caused by time of day or outside distractions. All horses were accurately weighed prior to the beginning of the study with a portable livestock scale (MTI500-WB, MTI Weigh Systems, Rhode Island). The study took place from May 2013 through January 2014.

Two square stalls measuring 3.1 m by 3.1 m with packed soil floors covered by rubber stall mats were used in this study (Figure 2). One stall was dedicated as a training stall while the other was dedicated the test stall. The training stall contained an unpainted wooden lever and feedbox located approximately 1.1 m off the floor in the middle of one wall of the stall. The lever was located approximately 6 cm to the right of the feedbox. The 2.5 cm^2 lever was mounted on a hinge so that approximately 30 cm projected into the training stall with an equal length projecting through a hole cut into the stall wall into the barn aisle where the researchers were located. The researchers could easily deliver the reinforcers and record responses from this position. The end of the lever in the aisle was weighted with a 10g weight to return the lever to its original position after being pushed by the horse. A fabric partition was used to block visual contact between horses and researchers.

The test stall contained two painted levers mounted approximately 1.1 m off the floor on either side of one wall approximately 0.9 meters from each opposing wall and separated by approximately 1.5 m. One feedbox was located in the middle of the wall approximately equidistant between the two levers. The lever located on the left side of the testing wall was 2.5 cm^2, painted white, and mounted on a hinge so that approximately 30 cm projected into the training stall and 30 cm was outside of the stall. Approximately 15 cm, above the lever, a white square (0.2 m^2) was tacked to the stall wall to further identify the lever as being white in color. The lever located on the right side was mounted identically to the white lever, but was painted black with a black square (0.2 m^2) tacked to the stall wall approximately 15 cm above the lever to further identify the lever as being black in color. Again, a fabric partition blocked visual contact between the horses and researchers. A diagram of the test stall and the discrimination test apparatus is shown in Figure 3.

The primary reinforcer used in the study was approximately 55 g of the horse's usual concentrate ration delivered by hand through a delivery chute from the barn hallway to the feedbox in the test stall. Efforts were made to dampen the sound of the primary reinforcer moving through the delivery chute and landing in the feedbox, but this process did make a discernible sound. The secondary reinforcer used was an auditory buzzer constructed from a commercial door buzzer (GE 45115 Wireless Window Alarms, Jasco Manufacturing Florence, Alabama). The smaller portion of the buzzer was attached to the lever so that a lever press delivered the secondary reinforcer, or if necessary, the researchers could activate it manually.

Each horse was trained with 30 lever presses with continuous reinforcement, daily for 3 consecutive days. Each day consisted of one trial. Each trail consisted of 30 lever presses. On the first day of training, horses were led into the training stall by a handler and were released to explore the stall. Once horses were comfortable with the stall, the shaping process began. All horses were shaped to push the lever to obtain the primary reinforcer paired with the secondary auditory reinforcer.

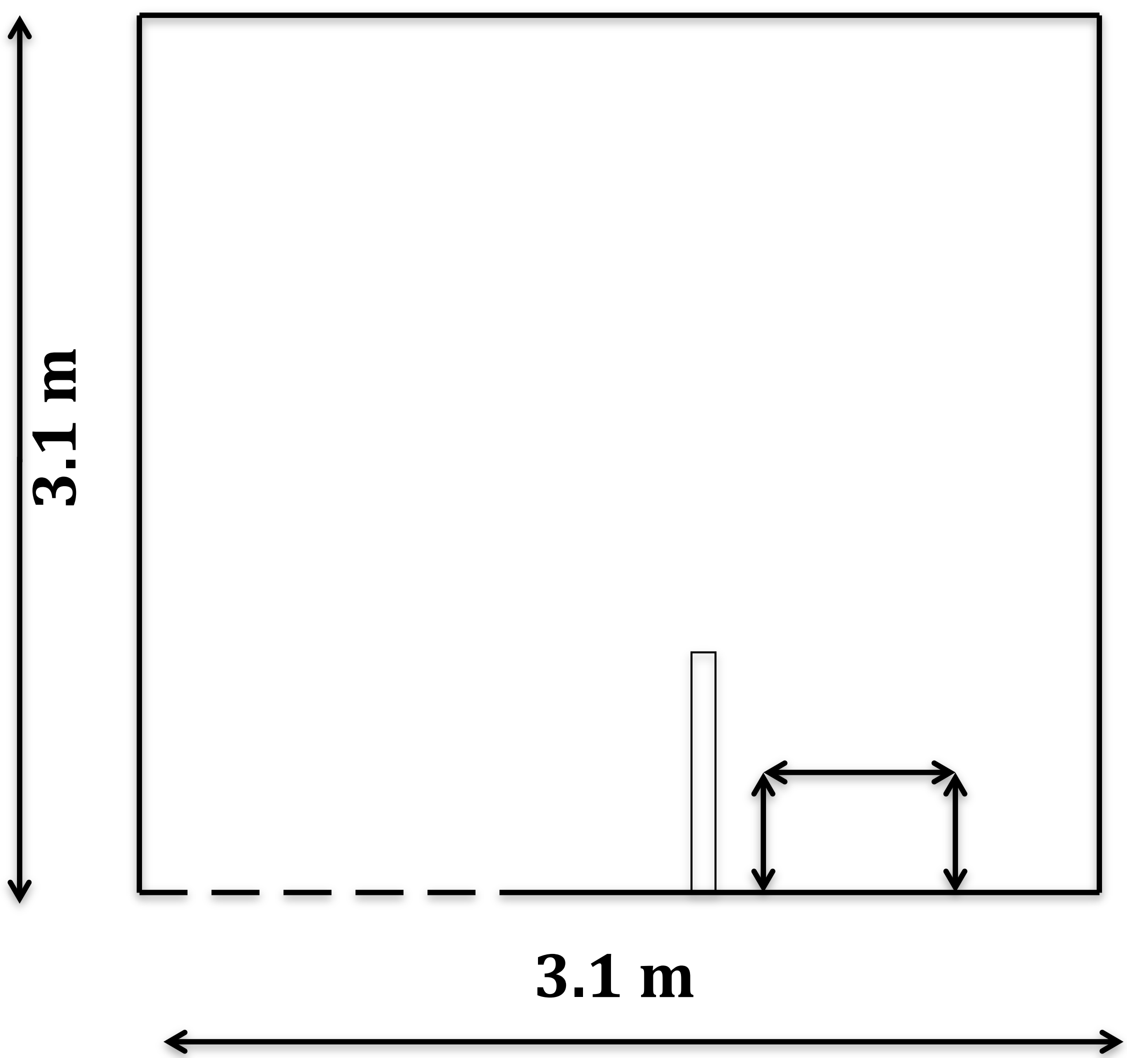

Figure 2. Discrimination training stall. Solid walls are designated by unbroken lines. Entryway is designated by a dashed line. Area of feed bucket is designated by double-sided arrow lines. Lever is designated by solid rectangle.

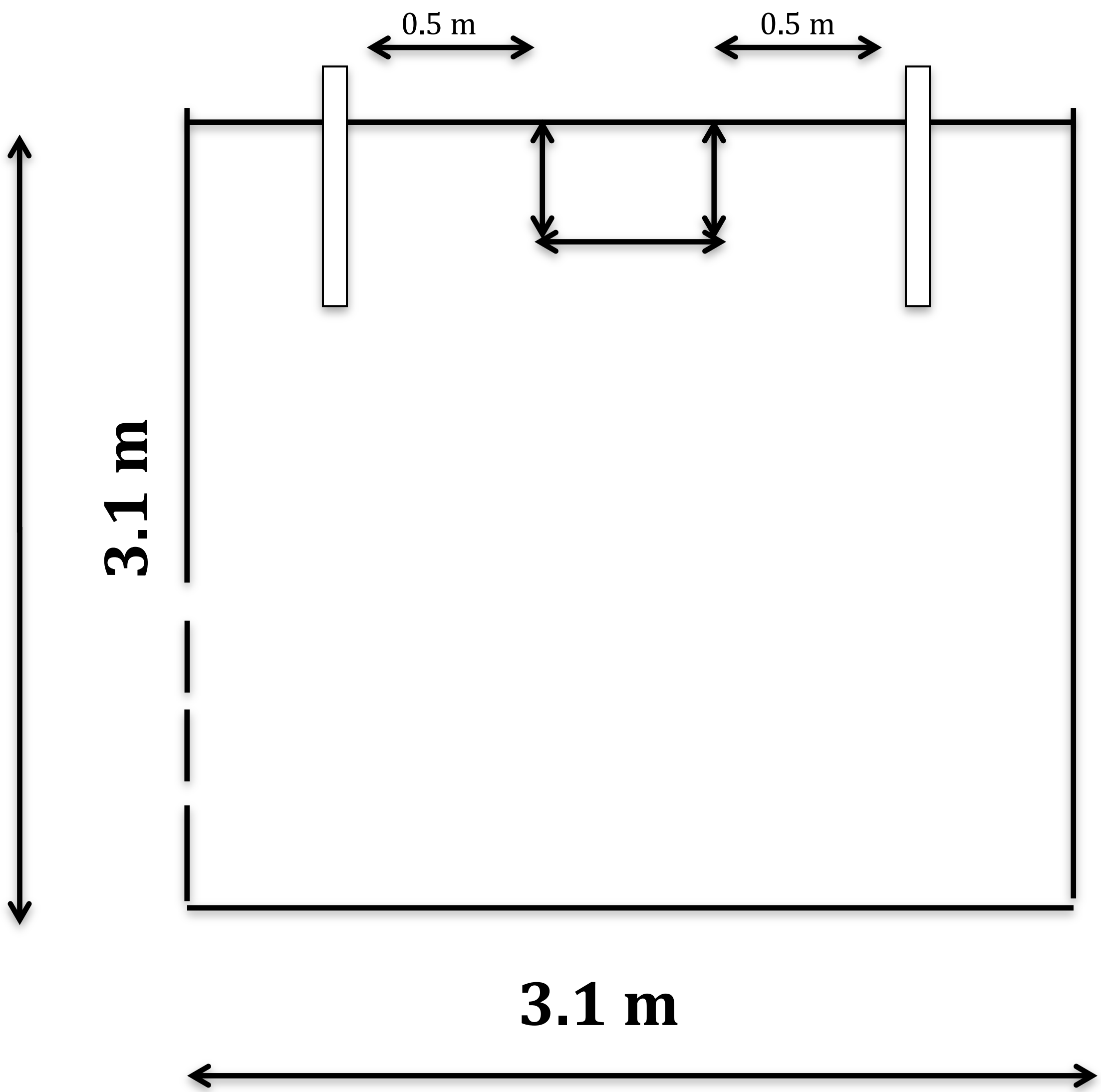

Figure 3. Discrimination testing stall. Solid walls are designated by unbroken lines. Entryway is designated by a dashed line. Area of feed bucket is designated by double-sided arrow lines. Levers are designated by solid rectangle.

During shaping, the handlers placed small amounts of concentrate on the lever and encouraged the horse to show interest in the concentrate. At points, the handler manually may have placed the horse's nose on the lever. Any movement towards the lever was reinforced with concentrate. Only when the lever was actually touched by the horse was the secondary auditory reinforcer introduced. Most horses learned the correct response within 30 minutes, and little aid was required by the handlers. This training continued for a total of three consecutive days for a total of three trials. Each trial was considered complete after 30 total lever presses by the horse. By day three of the training, no assistance from the handlers was required for the horses to lever press.

On the fourth day, trial 4, the horses were injected with either Ace (tranquilized) or saline (control) intramuscularly according to their previously assigned group. The horse was allowed to stand for 15 minutes to reach full pharmacological effect of the tranquilizer, which is the time recommended in the literature. After the 15-minute standing period, the horses were introduced to the test stall. The horse was led into the stall and faced away from the discrimination apparatus and released. A lever press of the previously assigned correct color (white or black) resulted in primary and secondary reinforcement, whereas the lever press of the wrong color resulted in no reinforcement. Time was recorded beginning at the first lever press of either color and stopped at the completion of the 30^{th} lever press or after 10 minutes had passed without an adequate lever press from the horse. There was no time limit as long as the horse completed a lever press within 60 minutes of the previous lever press. The trial was considered complete upon a completion of 30 total lever presses or failure of the horse to respond with a lever press for one hour. Numbers of correct and incorrect lever presses were recorded.

Fourteen days following trial 4, horses were tested to judge their memory recall of the white vs. black lever task. Horses were not injected with either Ace or saline control. The horses were led to the test stall and faced away from the discrimination apparatus and released. Horses then were allowed to lever press. Their originally assigned correct lever color was still designated as the correct lever, and correct lever presses were reinforced with feed and the buzzer. No reinforcement was delivered for a wrong color lever press. Horses were allowed to remain in the stall until 30 complete lever presses were recorded or until 1 hour passed without an adequate lever press by the horse. Time and number of incorrect or correct lever presses were recorded.

Approximately six-months later, horses were again re-tested for memory accuracy. The subject population was reduced to 25 mature horses upon retesting at six-months due to 10 horses being relocated where further testing was no longer possible. Horses were not injected with either Ace or saline. The horses were led to the testing stall and faced away from the discrimination apparatus and released. Horses were allowed to lever press without any reinforcement for any lever pressing response until extinction (10 minutes without an adequate lever press) or until completing 30 lever presses. Time and number of correct and incorrect lever presses were recorded for future analysis.

Statistical Analysis

Comparisons between the two groups were conducted using an independent two-sample t test. For the memory recall test, which occurred 14-days after trial 4 of the training sessions, correct responses and time to test completion were the dependent variables. During the six-month memory recall test, percent correct responses and time to test completion were analyzed because individual horses could exhibit differing numbers of responses prior to reaching extinction.

Results and Discussion

The three-day training trials resulted in all 35 horses learning the lever press task. The task was considered learned if the horses completed 30 lever presses on each training day. In the 14-day memory recall test, correct responses for control horses ranged from 28 to 30 correct responses whereas tranquilized horses ranged of 25 to 30 correct responses. Mean correct responses for control (29.5 ± 0.3) and tranquilized (28.9 ± 0.3) horses in the 14-day memory recall test did not differ ($P = 0.15$; Figure 4). Griffith (2006) also reported no significant differences in mean percent correct responses for control and tranquilized horses in a visual discrimination test (67.6 ± 2.0 and 69.8 ± 2.0, respectively; $P = 0.43$), which supports the results found in the present study. The first lever press of each horse was recorded as either correct or incorrect based on the previously learned color lever for each horse. In the 14-day trial, 15 of 18 (83%) horses in the Ace group correctly selected their assigned lever on the first press. Assuming that horses had a 50% probability of selecting the correct lever by chance, the probability of getting 15 or more correct responses on the first selection is 0.1%. The control horses had an 88% (15 of 17) accuracy in choosing the correct lever on their first lever press (a 0.3% probability). These results indicate that learning was facilitated equally between the groups, regardless of treatment.

The mean time, in seconds, for control and tranquilized horses to complete 30 lever presses in the 14-day memory/recall test (584 ± 54.8 and 544 ± 53.3, respectively) did not differ ($P = 0.44$; Figure 5). Tranquilized horses had a time range of 407 to 992 seconds compared to 324 to 1,319 seconds for control horses to complete 30 lever presses. Though there were no statistical differences in the mean times, it is interesting to note that the numerical time for the tranquilized horses was slightly faster than that of the control horses. This could indicate that Ace could promote learning and decrease trials needed to master a task. Ace possibly could allow the horses to better concentrate on the task and be less distracted by outside stimuli. Ace is most commonly used in situations in which the animal finds the conditions stressful, so the use of Ace in this study could have benefited the tranquilized group by reducing perceived stress by the test subject. Murphy et al. (1986) conducted research on the stress in the workplace and its effect on workplace accidents in humans. Their study reported that managing stress in the workplace reduced workplace accidents. So by reducing perceived stress in the discrimination test of the horses, the tranquilized horses might be expected to perform at a higher accuracy. Further research on this aspect in the present study would be beneficial.

The discrimination test at six-months detected no difference ($P = 0.55$) in mean percent correct responses for control and previously tranquilized horses (65.5 ± 5.1 and 69.1 ± 5.5, respectively; Figure 6). Mean percent correct responses ranged from 27.3 to 90.9 for previously tranquilized horses, while control horses ranged from 33.3 to 86.7.

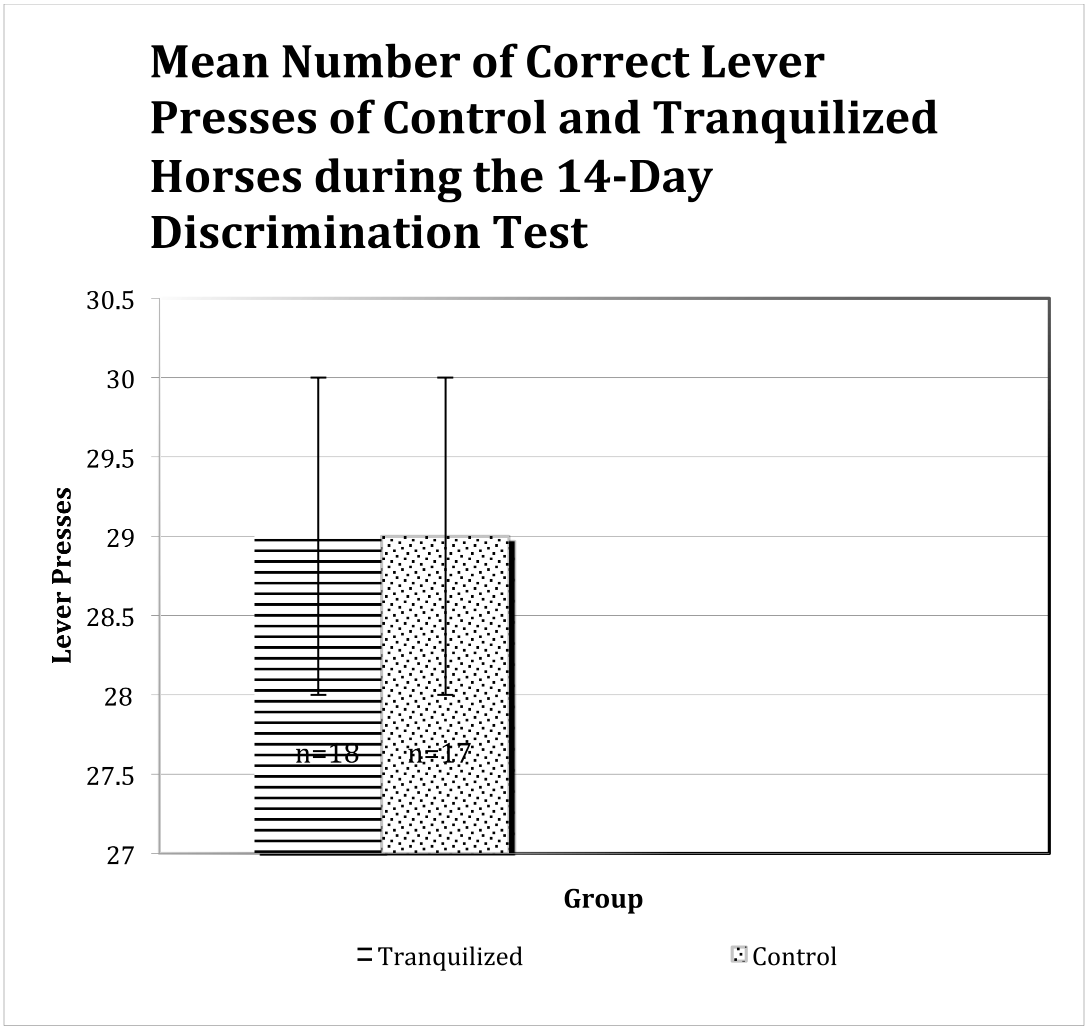

Figure 4. Mean number of correct lever presses for control and tranquilized groups did not differ (P = 0.15) during the discrimination test.

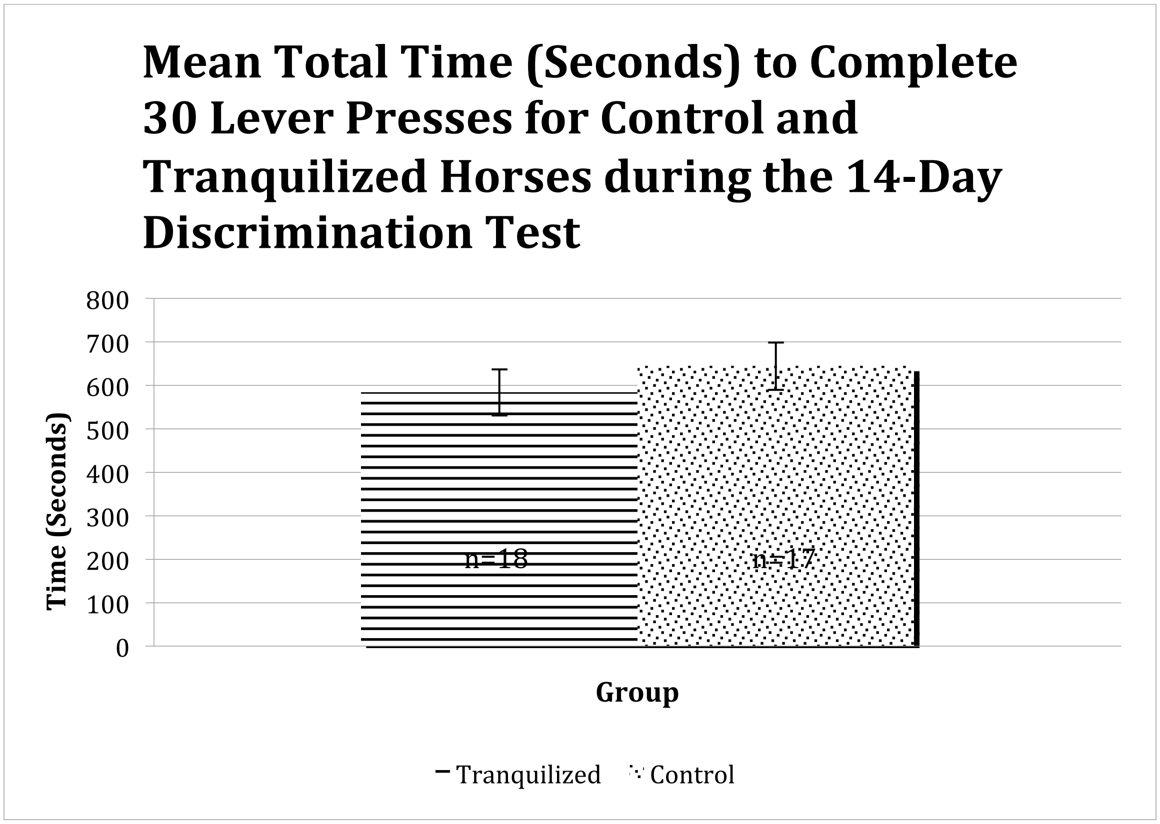

Figure 5. Mean total time for the tranquilized and control horses to complete 30 lever presses did not differ (P = 0.43) during the discrimination test.

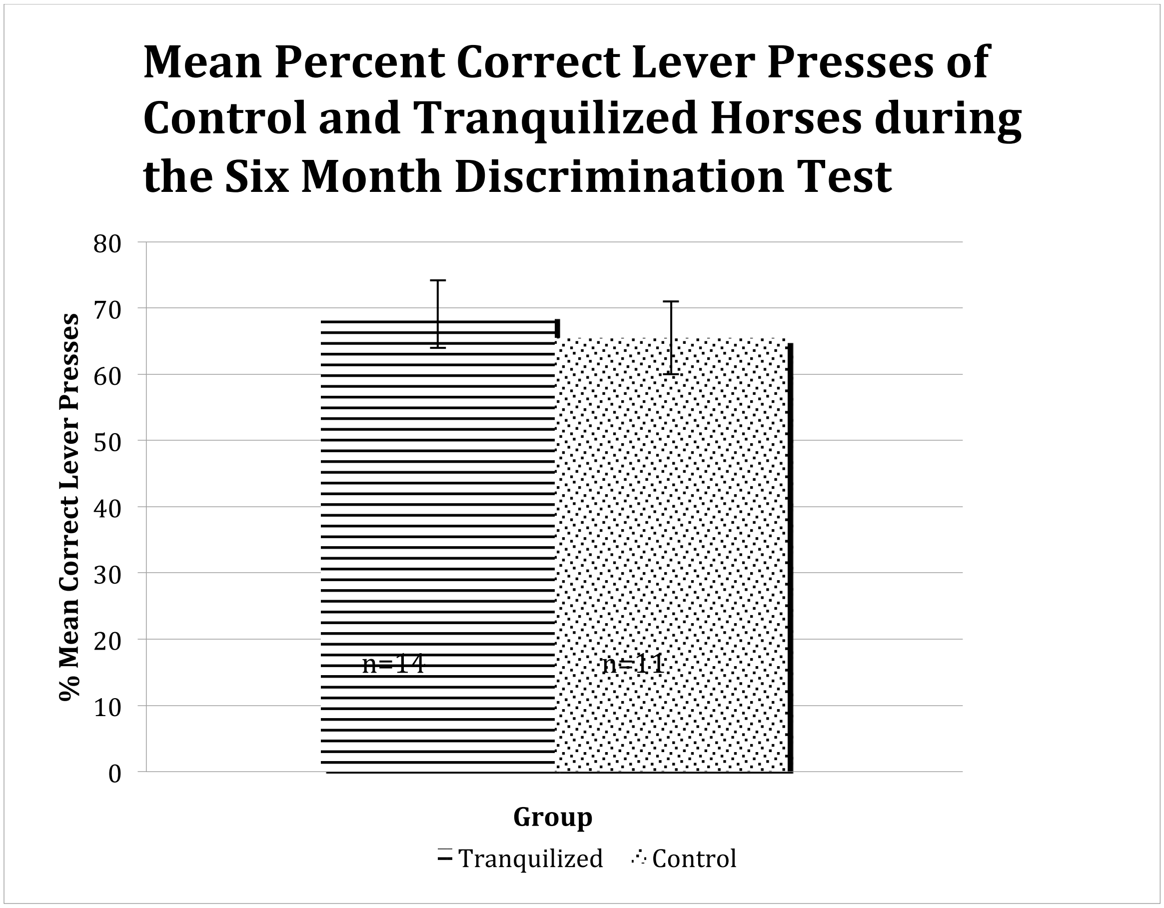

Figure 6. Mean percent of correct lever presses for the tranquilized and control horses did not differ (P =0.55) during the six-month discrimination test.

Eleven of the 14 Ace group horses correctly chose the correct lever on their first lever press in the six-month test. The probably of getting this many correct by chance is approximately 3%. Only 6 of 11 control horses correctly chose the correct lever on their first lever press, which is approximately around chance occurrence (50%). The higher accuracy of the tranquilized horses would suggest that they were more capable of recalling memory of the task. In combination with the mean percent correct responses of the tranquilized horses having no significant difference between the mean percent correct responses of the control horses, this information would further support the conclusions that tranquilized horses are capable of learning tasks and recalling them at later dates just as well, if not better, than control horses.

Mean times for the control and tranquilized horses to complete the 6-month memory recall test was 1082 seconds and 1023 seconds, respectively, and did not differ ($P = 0.77$). This is illustrated in Figure 7. Previously tranquilized horses had time responses from a minimum of 461 seconds to a maximum of 2190 seconds. Control horses had a range of 498 seconds to 1856 seconds. Again, there is no statistical difference of the mean time between the groups, but tranquilized horses continued to perform at a numerically faster time than those of the horses in the control group. During the extinction discrimination test, nine of 14 Ace horses did not complete 30 total lever presses during the extinction test, while eight of 11 control horses stopped responding before 30 lever presses.

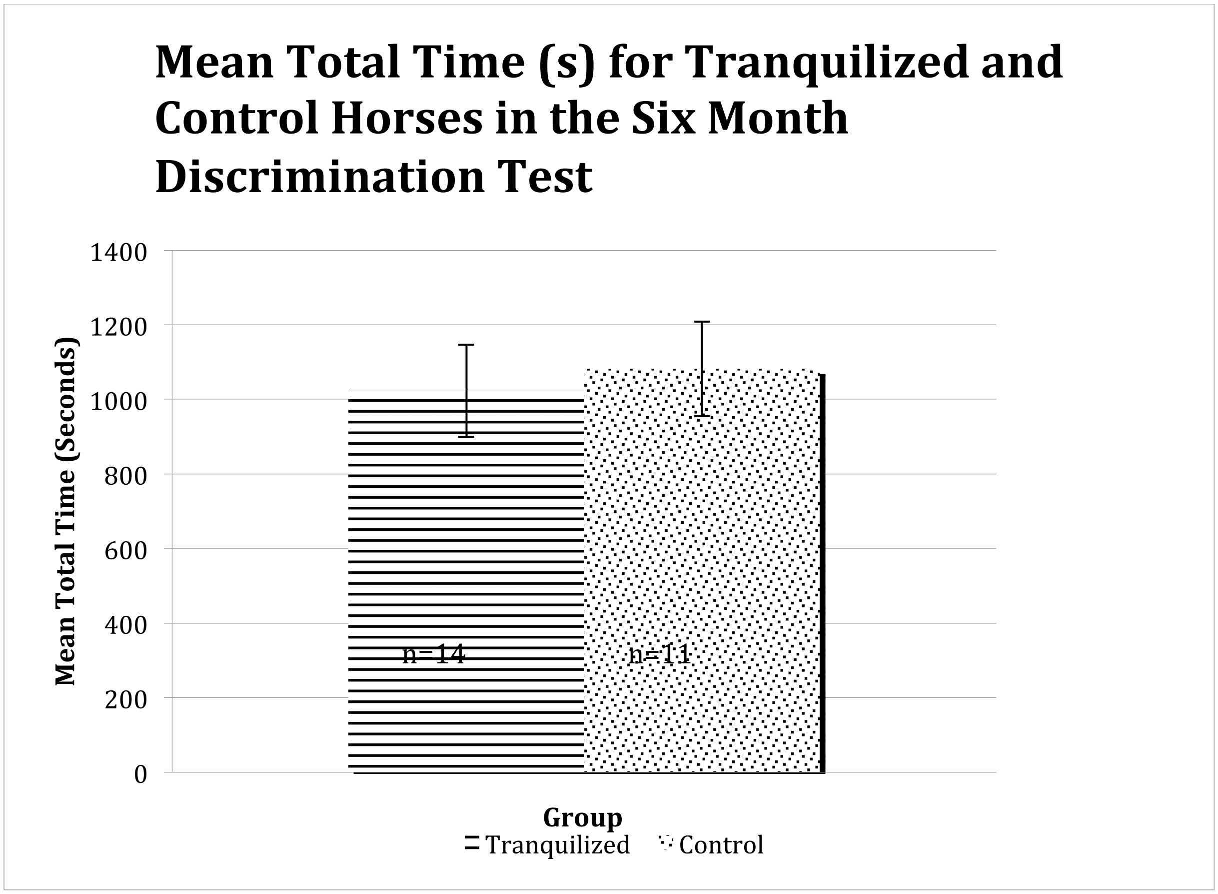

Figure 7. Mean total time (s) for the tranquilized and the control horses did not differ (P = 0.55) during the six month discrimination test.

Acepromazine maleate had no effect on the memory recall of horses used during this study. Tranquilized horses performed as well as the control horses in a discrimination test. These results were similar to the results found by Griffith (2006) in which learning performance on simple discrimination and spatial tests was similar in tranquilized and control horses. In this study, Ace was used in an elevated amount (0.088 mg/kg) to determine if the large amount of sedation would still result in learning. As a general rule, the dosage requirement in mg/kg of body weight decreases as the weight of the animal increases (ANADA, 2004). These studies suggest that Ace can be used as a training aid in appropriate situations, and the task can be appropriately learned.

Horses that were retested at 14-days and six-months after initial training showed memory recall ability. Tranquilized horses had similar memory recall performance on the discrimination test as those that were not. Therefore, Ace can be used as a training aid in appropriate situations, and the horses can be expected to recall that task at a later date. When appropriately used, a tranquilizer is beneficial in situations in which high levels of stress can be harmful to the horse or can be beneficial in situations in which there is risk to the horse or trainer. In situations, like loading into a trailer, which is both stressful and has the potential for harm, it might be advantageous to use Ace to teach the horse to load. The Ace allows the horse to remain calm and allows the handler to provide a safer environment for the horse and himself. This study suggests that using Ace would be beneficial in that the horse, while tranquilized, is still learning and will be able to recall the learning situation at a later date. This allows confidence in the trainer that when he tries to load that horse on the trailer at a later date, that the horse should be able to recall what it learned at the earlier date and be able to perform the task.

One limitation of this study is the simplicity of the of the two-lever discrimination test. It would have been interesting to do a testing trial with a three-lever discrimination test. This would allow a greater confidence in the actual learning ability of the horse to discriminate between three stimuli instead of two. To further differentiate the learning ability, the colored levers could have been rearranged so that their position was not fixed in the same location for each trial.

In the present study, it could have been beneficial to have the horses train to extinction in the 14-day testing instead of allowing positive reinforcement. The extinction test may have more accurately exposed the learning ability of the horses. It is possible that the use of the positive reinforcement on the 14-day test resulted in more training, which affected results of the six-month test. Reinforcements during the 14-day trial increased the time of overall learning. If an extinction test had been run on the 14-day trial, learning would have no longer been reinforced, and recall during the six month test would have been based off of the training days only instead of training and testing days together. Without the 14-day reinforcement, true learning ability and memory recall would have been more evident on the six-month trial.

Another limitation of the simplicity of the two-lever discrimination trial is the possibility of horses having a side preference of either the left or right lever. Most animal species exhibit left-right asymmetry in their body plans and show a strong bias for one handedness over the other (Wood, 1997). Lateralization has been illustrated in horses, but the validation of right-or left-handedness is small (McGreevy and Rogers, 2005). Murphy et al. (2005) reported a positive correlation between direction and gender in horses. They concluded that male horses are more likely to exhibit left lateralized responses and female horses exhibited more right lateralized responses (Murphy et al., 2005). In hindsight, it would have been interesting to record first choice lever pressing before any training/learning had begun to further test the results reported by Murphy et al. (2005). Further research into this subject could benefit owners who are choosing horses for specific tasks such as herding or barrel racing, which can be direction oriented.

Other limitations of the study included the inability to control outside stimuli throughout portions of the testing of the study. There were constant disturbances that could not be isolated from the training stall that could have contributed to the concentration of the study horses. It would have been beneficial to have an isolated stall, away from outside stimuli that would have allowed complete concentration of the study horses on the discrimination tasks. It is possible that the tranquilized group had an advantage over the control group because of the calming effects of Ace. The control horses did not have the added benefit of the tranquilizer, so it is possible that outside interference could have affected this group at a greater intensity than the tranquilized group. It would have benefited the study to have complete control over outside influences so that neither group had a possible advantage over the other treatment group. When training, it is ideal to have the horse's complete attention on the trainer and the task at hand. Real world training situations has many distractions that may hinder learning. It would be beneficial to use Ace in situations in which undivided attention from the horse is required because it allows the trainer to maintain the horse's attention because of the calming effect of the sedative, resulting in better learning.

Some of the test subjects may have lacked motivation for performing the test because they were being used as training horses in summer camps (14-day trial) and as equestrian team horses (six-month trial) during the duration of the study. It is hard to limit outside stimulus and feed intake with the amount of handling and work outside of the study. Limiting food intake before testing would have been beneficial in aiding the horse's motivation to perform. The horses were on a regular feeding schedule that could not be altered due to workload outside of the study. Most mature horses are food oriented, making this drive easy to manipulate. Horse owners often feed a low roughage diet because of convenience and the lower production of manure (Elia et al., 2010). Elia et al. (2010) determined that horses on a low forage diet have a higher motivation for a hay food reward over a concentrate or pellets as a food reward, but that the pellet reward still provides a source of motivation. When using reinforcement, a subject is more likely to respond if the reward is something highly sought after. Since the horses are feed low forage diets, it would fit that the horse would work harder for the scare resource. The present study used a pellet concentrate as a food reward because of convenience of delivery and because of decreased chewing time. Elia et al. (2010) determined that a horse spends more time chewing a hay diet (43,476 chews/day) than a pelleted diet (10,036 chews/day).

The quick absorption rate, rapid calming effect, and popularity of use among horse owners make Ace an ideal tranquilizer for use in this study. This study required a pharmacological agent that was relatively fast acting to reduce stress on the horse and to allow the study to move at a fast pace. Ace also was chosen because of its general safety and low risk of side effects in most horses. It is a tranquilizer that is readily available, making it a tool that is widely used by horse owners and trainers. Using Ace as a tranquilizer in this study made results obtained more applicable to common training practices utilized with horses

Because Ace often is used to help make horses more tractable, it would be advantageous to handlers if Ace were suitable for use in learning procedures as indicated by this study. Ace allows less skilled handlers of fractious horses to perform mildly aversive procedures (e.g., trailer loading or clipping) while allowing the horse to learn to tolerate these procedures and recall this information at a later date.

It would be interesting to conduct a further review into state-dependent learning while using Ace as the tranquilizer. While this study suggests that horses tranquilized with Ace have the same ability to recall learned tasks as control horses, it would be beneficial to understand whether testing under the same state as the learning would increase recall ability. Lowe (1986) in research with humans determined that behavior learned in one drug state is better remembered when retention is tested in the same drug state. Lowe's findings would suggest that horses would better recall learned task in the same state that the task was originally learned. This distinction, if further studied, would be beneficial to horse handlers in helping them to determine whether or not the use of Ace would be beneficial or practical in the training situation.

Implications

Horses designated to the Ace group and control group were not significantly different in their recall ability of a discrimination task. These results indicate that Ace, at the doses used in this study as a tranquilizer, does not inhibit nor facilitate learning. It suggests that horses have the same capability to learn while under the influence of Ace as their counterparts not under the influence of Ace. This study concluded that horses could recall a learned discrimination task at both 14 days and six months after completing the initial learning trial while tranquilized. This implies that Ace can be used as a learning tool with long term recall.

The availability and low economic cost of Ace can lead to the misuse of the product. While Ace is a safe drug when used properly, improper use and overdose can result in damage to the horse. The most common being the permanent penile protrusion of male horses. It is important, as with all pharmaceutical products, to follow all dosage directions and to consult a veterinarian before use. Horses in sales and shows have been known to be injected with Ace to misrepresent the true temperament of the horse and make the horse more likely to sell at a high price or place in a competition. This type of unnecessary administration of Ace can lead to problems with animal welfare and animal ethics. Because Ace makes a horse more tractable without interfering with learning performance, an inadequate handler may endanger a horse by attempting to force an un-tranquilized horse to perform an aversive procedure, potentially inflicting pain or causing injury to the horse, while a tranquilized horse will be more tolerant of the aversive procedure (Griffith, 2006) avoiding discomfort or injury.

The appropriate use of Ace, as a training aid, is an important task held by horse handlers and trainers. It is the job of the handler to keep the horse's welfare in mind when using pharmaceutical tools. While Ace does not inhibit learning or memory recall, it should only be used in appropriate situations. Ace, when used appropriately, can be a valuable tool to the horse handler and help contribute to safety of the horse and handler. Continuing researching into pharmaceutical effects on the behavior of horses will benefit the horse community and will allow horse handlers and trainers more control over horses resulting in a safer training regimen.

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Appendices

Appendix 1. Test subjects, treatment group, correct number of lever presses and total time for discrimination test at 2 weeks

Horse	**Ace or Control**	**Amount Correct**	**Total Time**
Ben	control	30/30	23:58
Pru	control	30/30	0:58
Logic	ace	30/30	1:58
Glenda	control	30/30	2:58
Taylor	ace	30/30	3:58
Lena	control	29/30	4:58
Beau	control	30/30	5:58
Nick	ace	29/30	6:58
Fancy	ace	29/30	7:58
Hickory	ace	30/30	8:58
Pablo	ace	30/30	9:58
Quinten	control	29/30	10:58
Wendell	ace	29/30	11:58
Clover	control	29/30	12:58
Sparky	ace	26/30	13:58
Hercules	control	30/30	14:58
Toby	ace	30/30	15:58
Cantano	control	28/30	16:58
Max	ace	29/30	17:58
Cash	ace	30/30	18:58
Sandrik	ace	28/30	19:58
Davin	ace	30/30	20:58
breezy	control	29/30	21:58
Charlene	ace	28/30	22:58
Mr.Big	control	30/30	23:58
Vancouver	control	30/30	22:58
Johnny	control	30/30	1:58
Changoo	ace	25/30	2:58
Clifford	ace	30/30	3:58
Skip	control	30/30	4:58
Tula	ace	28/30	5:58
Money	control	30/30	6:58
Chevy	control	28/30	7:58
Big Bird	ace	30/30	8:58
Calvin	control	30/30	9:58

Appendix 2. Test subjects, treatment group, number of correct lever presses and total time for discrimination test at six months

Horse	Ace or Control	Right	Wrong	Time
Chevy	Control	11	7	19:18
Bird	Ace	18	2	13:11
Calvin	Control	8	4	22:59
Tula	Ace	3	8	36:30:00
Clifford	Ace	24	6	11:40
Skip	Control	5	2	13:09
Changoo	Ace	2	1	9:47
Mr. Big	Control	23	7	8:18
Charlene	Ace	5	2	10:28
Sandrik	Ace	16	14	12:37
Davin	Ace	14	9	17:50
Max	Ace	27	3	23:47
Cash	Ace	8	4	14:18
Cantano	Control	7	9	26:52:00
Sparky	Ace	9	21	16:08
Hercules	Control	26	4	12:31
Wendell	Ace	3	1	7:41
Clover	Control	6	6	13:31
Pablo	Ace	15	2	19:56
Quinten	Control	2	4	14:00
Beau	Control	5	2	14:02
Taylor	Ace	23	7	26:39:00
Glenda	Control	26	4	30:56:00
Logic	Ace	20	2	18:18
Pru	Control	8	3	22:46

Appendix 3. Horses lost in 6-month discrimination test due to relocation

Horse	**Group**
Ben	Control
Lena	Control
Nick	Ace
Fancy	Ace
Hickory	Ace
Toby	Ace
Breezy	Control
Vancouver	Control
Johnny	Control
Money	Control