THE EFFECTS OF NMDA ANTAGONISTS ON LATENT INHIBITION VIA CHRONIC ADMINISTRATION OF MK-801

By

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A thesis submitted to the faculty of Radford University
in partial fulfillment of the requirements for the degree of
Master of Arts in the Department of Psychology

December 2013

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ABSTRACT

Evidence shows the N-methyl-D-aspartate (NMDA) receptor for the neurotransmitter glutamate plays an important role in regulating learning and behavior. These receptors are critically involved in the establishment of long-term potentiation (LTP). LTP is a widely studied cellular mechanism of learning and memory that has been observed in the Hippocampus and other areas of the brain. Researchers have investigated the role of LTP and NMDA receptors in many behavioral learning and memory tasks. One study showed the NMDA antagonist MK-801 ((+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclo- hepten-5,10-iminemaleate) produced deficits in place learning and spatial strategies in the Morris water maze (Robinson, Crooks, Shinkman, & Gallagher, 1989). Another hippocampal-dependent task that is commonly studied is called latent inhibition. There are many discrepancies in past literature regarding the nature of MK-801's effects on latent inhibition. The purpose of this study was to examine how the NMDA antagonist MK-801 affects a within-subjects, appetitive latent inhibition task. Long-Evans rats received subcutaneous injections of either a low (0.05 mg/kg) or high (0.1 mg/kg) dose of MK-801, or saline. The subjects received 1 day of magazine training, 4 days of non-reinforced exposure to either a clicker or a white noise, and 4 days of conditioning in which the preexposed stimulus and a novel stimulus were paired with the delivery of a food pellet. Conditioning days were recorded and scored for 6 different behaviors occurring just before and during each conditioned stimulus (CS) presentation. Results indicated that rats displayed significantly more conditioning behavior to the clicker CS than the white noise CS independent of which stimulus was preexposed. Evidence suggested the clicker could possibly have unconditioned aversive properties. Nevertheless, rats showed more rapid acquisition to the novel clicker when preexposed to

the white noise, which signified a latent inhibition effect. MK-801 neither disrupted nor enhanced the latent inhibition effect; therefore, NMDA receptors were not critical for latent inhibition.

Keywords: NMDA receptors, NMDA antagonists, MK-801, latent inhibition

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DEDICATION

To my loving parents Paul and Susan, and my wonderful brother Matthew, whose continuous encouragement and support throughout my academic career means the world to me. I am truly blessed to have such an amazing family; I dedicate this to you.

ACKNOWLEDGEMENTS

A special thanks to my advisor Dr. Jeffrey Willner and to undergraduate researchers Brittany Nipper, Danny Ramirez, Emily Aquilina, Adam Terrell, Erika Carr, and Dante Lavender who devoted their time an effort toward the completion of this research project.

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CHAPTER 1. INTRODUCTION

The N-methyl-D-aspartate (NMDA) receptor for the neurotransmitter glutamate has attracted considerable attention over the last 30 years. Interest in NMDA receptors derives from a number of sources, including evidence that these receptors could play a role in the brain damage caused by strokes (Suzuki, Takagi, Nakamura, Hashtmoto, & Umemura, 2003). There is also a growing body of evidence implicating NMDA receptor dysfunction in schizophrenia (Weiner & Arad, 2010). Much of the interest in NMDA receptors, however, stems from demonstrations that these receptors play a critical role in the induction of some forms of long-term potentiation (LTP). LTP refers to the observation that high-frequency stimulation of the inputs to a neuron can cause a long-lasting increase in the ability of those inputs to activate that neuron, in other words, an enhancement of synaptic efficacy. LTP was first demonstrated in the hippocampus by Bliss and Lømo in 1973, and has a number of characteristics that make it an attractive model for how information might be stored in the mammalian nervous system. Several of these characteristics include: its relatively long duration, its prominence the hippocampus, which is known to be critical for certain types of learning and memory, and its specificity to activated synapses (Morris, 1989). These features have served to make LTP in the hippocampus, and other brain regions, a widely studied model for investigating the cellular mechanisms of learning and memory (Eichenbaum, 2008).

Studies have shown that NMDA receptors in the dentate gyrus and CA1 areas of the hippocampus play a significant role in the induction of LTP. Harris, Ganong, and Cotman, (1984), for example, demonstrated that blocking NMDA receptors would prevent the establishment of LTP in the CA1 region of the hippocampus without

impairing normal synaptic transmission in that area. Conversely, Muller, Joly, and Lynch (1988) demonstrated that administration of NMDA antagonists had little effect on the magnitude of LTP once it had been established. It thus appears that NMDA receptors are critical for triggering LTP, but play little role in the maintenance or expression of LTP. Instead, the maintenance and expression of LTP appears to depend on changes that occur at the so-called AMPA receptor for glutamate (Collingridge, 1992).

Insight into the mechanisms underlying LTP has led to studies examining whether these same mechanisms also play a role in behavioral learning and memory. One way that researchers have approached this notion is by examining the effects of NMDA antagonists on animals' ability to acquire learning tasks that depend on the hippocampus. The hippocampus maintains a well-documented role in place learning and navigation in animals (Jarrard, 1993; O'Keefe & Nadel, 1978); therefore, a number of studies have looked into how NMDA antagonists affect rats' ability to acquire place learning tasks, such as the Morris water maze (Morris, 1984) and the radial arm maze (Olton, Becker, & Handelmann, 1979). By and large, the results of such studies have shown that administration of NMDA antagonists, prior to training, will disrupt the acquisition of these sorts of spatial learning tasks. Morris, Anderson, Lynch, and Baudry (1986), for example, showed that the competitive NMDA antagonist AP5 (aminophosphonovaleric acid) caused deficiencies in place learning in the Morris water maze without impairing rats' ability to learn a visual discrimination in the maze. Similarly, Robinson, Crooks, Shinkman, and Gallagher (1989) showed that the noncompetitive NMDA antagonist MK-801 (dizocilpine; (+)-5-methyl-10, 11-dihydro-5H-dibenzo [a,d]cyclo- hepten-5,10-iminemaleate) caused a deficit in the acquisition of place, but not "cue" learning, in the

Morris water maze. A number of other studies have produced comparable findings (Carmanos & Shapiro, 1994; McLamb, Williams, Nanry, Wilson, & Tilson, 1990; Morris, Halliwell, & Bowery, 1989; Shaprio & Caramanos, 1990; Sharpiso & O'Connor, 1992). Although sensorimotor deficits caused by NMDA antagonists may contribute to deficits in acquisition of spatial tasks in some instances (Cain, Saucier, & Boon 1997), other evidence suggests that NMDA antagonists do indeed have a selective effect on spatial learning (Marr & Willner, 1999; Mackes & Willner, 2006, Pixley, Peddy & Willner, 2004). There is good support, then, for the idea that NMDA receptors play an important role in spatial learning.

Researchers have also examined the effects of NMDA antagonists on latent inhibition, another learning phenomenon thought to depend on the hippocampus. Latent inhibition refers to the observation that repeated, non-reinforced exposure to a stimulus retards conditioning of that stimulus when it is subsequently paired with an unconditioned stimulus (Lubow & Moore, 1959). A number of studies examining the effects of hippocampal lesions on latent inhibition have shown that latent inhibition is greatly diminished or abolished after hippocampal damage (Kaye & Pearce, 1987; Schmajuk, Lam, & Christiansen, 1994; Solomon & Moore, 1975), and that the hippocampus is intimately involved in modulating the "associability" of stimuli (Schmajuk & Moore, 1988; Solomon, 1979). These findings suggest that administration of NMDA antagonists should impair latent inhibition. However, the results obtained in studies examining this question have been less clear-cut. Some studies have indeed obtained deficits in latent inhibition (Aguado, San Antonio, Perez, del Valle, & Gomez, 1994), while others have found no effect of antagonists on latent inhibition (Robinson,

Port, & Stillwell, 1993; Tenn, Kapur, & Fletcher, 2005; Weiner & Feldon, 1992). At the same time, some have even found a more "persistent" latent inhibition in rats given NMDA antagonists (Gaisler-Solomon & Weiner, 2003; Lipina, LaBrie, Weiner, & Roder, 2005). There thus appears to be a discrepancy between the effects of hippocampal lesions and the effects of NMDA antagonists on latent inhibition.

One possible explanation for the discrepancy between hippocampal lesion and NMDA antagonist studies is that the hippocampal involvement in latent inhibition is not what it was believed to be. The majority of early studies demonstrating deficits in latent inhibition following hippocampal lesions utilized electrolytic/aspiration lesions, which can damage not only the hippocampus, but fibers of passage and adjoining brain areas. Studies employing more recent neurotoxin lesioning techniques, known to produce less collateral damage (e.g., Jarrard, 1989), often harvest results that differ from those obtained with older lesion methodologies, which may shed light on the ambiguity of hippocampal involvement in latent inhibition.

Honey and Good (1993) examined the effects of neurotoxin lesions of the hippocampus on latent inhibition using an appetitive conditioning paradigm. They found that latent inhibition was intact in rats with ibotenic acid lesions of the hippocampus; however, neurotoxin lesions did disrupt the context specificity of latent inhibition. Context specificity refers to the observation that animals only effectively show latent inhibition when stimulus exposure and subsequent conditioning take place in the same environment (Lubow, Rifkin, & Alek, 1976). Honey and Good (1993) found that changing contexts between exposure and conditioning did not disrupt latent inhibition in rats with hippocampal lesions; rather, latent inhibition for the preexposed stimulus

generalized across contexts, but was disrupted in rats that had received control operations. This result suggests that the hippocampus is not so much critical for the basic latent inhibition effect as it is critical for the context specificity of the effect.

The present study examined the effects of the NMDA antagonist MK-801 on latent inhibition in rats using a within-subjects, appetitive learning task, much like that developed by Bonardi, Bartke, Boweles, de Pulford, and Jennings (2010) for mice. The use of appetitive conditioning is desirable for humane purposes, and for establishing the generality of results obtained in past studies, the majority of which used an aversive conditioning technique. Similarly, the use of a within-subjects procedure is advantageous because of the greater sensitivity of such designs when compared to the between groups designs typically employed in behavioral studies of latent inhibition.

In this study, 27 male Long-Evans rats received subcutaneous injections of saline or 1 of 2 doses of MK-801 (0.05 mg/kg or 0.1 mg/kg) prior to stimulus exposure, magazine training, and conditioning sessions in which the preexposed stimulus and a novel stimulus were presented. In this case, latent inhibition would be revealed by more rapid acquisition of conditioned responding to the novel stimulus than to the familiar, preexposed, stimulus. Use of a range of doses of MK-801 is appropriate in order to establish a dose-response curve for whatever effects are obtained, additionally, higher doses of the drug (e.g., 0.1 mg/kg) can bring about sensorimotor effects that could potentially lead to a general disruption of behavior. This study should establish whether NMDA receptors are critical for the basic latent inhibition effect, or whether further studies examining the context specificity of latent inhibition in animals given NMDA antagonists need to be undertaken.

CHAPTER 2. METHOD

Subjects

27 male Long-Evans rats, 103-128 days old, were studied to obtain reasonable power ($\beta = .8$) in order to detect medium - to large - sized effects. All rats were group housed in either plastic hanging tubs (44 cm L x 22 cm W x 20.5 cm H) or metal cages (74 cm L x 57 cm W x 25 cm H). The lights were on between 0700-1900 hours daily. All testing was conducted during the light part of the cycle. All rats received ad libitum access to food and water, prior to food restriction, and were handled and weighed on a daily basis. Data from 3 rats were excluded due to an improper conditioning protocol.

Food Restriction

Rats were moved to individual housing in stainless-steel hanging cages (25 cm L x 18 cm W x 18cm H) just before the start of food restriction. All rats were placed on restricted food access 2 weeks prior to the beginning of the study and maintained at 90% of their average free feeding body weight during the study. In order to determine their free feeding body weights, each rat was weighed once a day, for 7 consecutive days, while receiving ad libitum access to food and water. The average weight from those 7 days was then used as the free feeding body weight. Rats were then placed on food restriction where only 5-10 grams were given each day until their weight had dropped to about 90% of the free feeding weight. Subsequently, each rat received enough food to maintain its weight within 10 grams of its 90% weight.

Drug Preparation and Administration

Rats were randomly assigned to 1 of 3 treatment groups (n=8): Vehicle (isotonic saline), 0.05 mg/kg of MK-801, or 0.1 mg/kg of MK-801. Aliquots of 100 µl of MK-801

were kept frozen until needed. Prior to each session MK-801 was prepared by thawing an aliquot and dissolving it in a sterile saline, 900 µl of saline was used for the low dose and 400 µl of saline was used for the high dose. Rats were injected based on their weight. Their weight was measured in grams and multiplied by 0.5 then divided by 1000 to produce doses with an injection volume of 0.5 ml/kg. MK-801 and vehicle injections were administered subcutaneously 20 minutes prior to the beginning of each day's sessions.

Apparatus

Three identical conditioning chambers (Coulbourn Instruments) were used in the study (Figure 1). Treatment groups were counterbalanced among the chambers (see Appendix 1). Each chamber was a 12 sided cylinder (41.5 cm x 25 cm) consisting of 12 stainless steel panel walls (25 cm L x 7 cm W) with a transparent polycarbonate ceiling (44.5 cm diameter). Mounted in the center of one wall was a feeder with an opening measuring 3 cm x 4 cm x 2.3 cm. The feeder was connected to a pellet dispenser from which 45 mg pellets (Noyes Precision Food Pellets, Formula A/1) were delivered. The feeder was located 1.5 cm above a stainless steel grid floor with a removable metal waste pan, containing wood shavings, beneath it. Feeder entries were detected when an infrared photo beam located across the opening of the feeder was interrupted. A house light (12-W bulb in metal housing) was mounted at the top of the panel wall directly across from the feeder. Modules for delivering auditory stimuli (white noise and clicker) were located on opposite panels, halfway between the feeder and house light, at right angles to them. One wall had a speaker that delivered a 75 dB white noise, while another wall had a relay that presented 2 Hz clicks at 75 dB. All events in the chambers were controlled by a

personal computer located in an adjacent room running Graphic State 3.03 software (Coulbourn Instruments). Low-light video cameras mounted directly above the chambers were used to record all sessions on a digital video recorder for later analysis. To remove olfactory cues between individual sessions, the conditioning chambers were cleaned with 10% (water/vinegar) solution and wood shavings were discarded and replaced.

Figure 1: Coulbourn operant conditioning chamber

Latent Inhibition Procedure

Latent inhibition training took place over 9 consecutive days, consisting of the following phases: magazine training, preexposure, and conditioning.

Magazine Training. There was 1 day of magazine training in which rats learned to stick their nose in the feeder to receive a food pellet. During magazine training there were 49 pellet deliveries over a 40 minute period. The number of feeder entries was recorded before, during, and after the delivery of pellets. If the rat did not learn to utilize the feeder a second day of magazine training was added to the protocol.

Preexposure. Each rat received 4 sessions of exposure to an auditory stimulus prior to conditioning; half of the rats were exposed to a white noise (n = 12), and the other half to a clicker stimulus (n=12). Each session consisted of 20, 10 second exposures to the stimulus with a variable intertrial interval of 70-100 seconds between successive stimulus presentations. Each preexposure session lasted approximately 50 minutes.

Conditioning. The rats received 4 sessions in which the 2 auditory stimuli, the clicker and the white noise, were presented on separate trials and paired with food delivery. Each trial consisted of a 10 second presentation of one of the auditory stimuli with delivery of two 45 mg food pellets at the end of the stimulus presentations. The rats received a total of 15 trials with each stimulus over the course of a session, randomly ordered, with the constraint that there could be no more than 3 consecutive trials with a given stimulus. During conditioning sessions, the mean duration of the intertrial interval increased to 100-130 seconds between trials. Each conditioning session lasted approximately 65 minutes.

Coding Behavior

Video recordings were later analyzed for behaviors occurring during the 10 seconds preceding each CS presentation (preCS period) and during the 10 second CS presentations (CS period). Behavior was sampled every 2 seconds during preCS and CS periods and scored in 1 of 6 categories adapted from Holland (1997). The only difference was that freezing/standing motionless, swinging from the hole in the ceiling, balancing by placing paws on the ceiling, and sleeping was added to the Other category, as seen in Table 1.

Table 1: Behavior categories (adapted from Holland, 1997).

Perambulate:	Change in position involving all four feet, including walking across chamber, circling, and/or jumping suddenly to another position; often accompanied by sniffing.
Rear	Standing on hind legs with both forepaws off the grid floor, usually (not always) stretching to full extent, forepaws usually (not always) on top of side walls of chamber, often pawing walls; may be accompanied by sniffing or slow side-to-side movement of head. Does not include grooming movements, even if performed while standing on hind legs.
Magazine	Standing motionless in front of food magazine with nose or head within magazine, sometimes (rarely) gnawing on edges of magazine opening.
Head-jerk	Short rapid horizontal and/or vertical movements of the head, usually oriented toward food magazine; hindquarters motionless. Infrequently occurring with rear: In those cases only head-jerk scored.
Head-jerk/hind	Head-jerk plus movement of hind-quarters, either side-to-side or forward-backward. Simultaneous display of head-jerk and perambulate (rare) also scored as head-jerk/hind.
Other	Grooming head, body or tail; scratching; gnawing grid bars; standing motionless with head above or between grid bars; lying with abdomen on grid floor; sniffing (provided rat not also per- forming one of above behaviors). Additionally, freezing/standing motionless, swinging from the whole in the ceiling, balancing with paws on the whole in the ceiling, and sleeping.

Statistical Plan

The primary data for analysis came from observations of behavior during the 4 conditioning days. Behavior was observed and scored in one of Holland's (1977) behavioral categories every 2 seconds during the 10 second preCS and CS periods, producing 5 preCS and 5 CS observations per trial. These observations were then used to calculate the percentage of observations on which a given behavior occurred during preCS and CS periods for each auditory CS (clicker and white noise). These percentages were analyzed using repeated measures or mixed-model Analyses of Variance (ANOVAs) as appropriate. Significant interactions were assessed using one-way ANOVAs and post hoc comparisons as needed. In cases where the assumption of sphericity was violated, reported *p*-values were adjusted by the Greenhouse-Geisser correction of the *F*-test. All data was analyzed using SPSS version 19.0 software, StatView 5.0, and Microsoft Excel 2010.

CHAPTER 3. RESULTS

Identification of Conditioning Behavior

Preliminary analyses were completed to identify which behaviors (Perambulate, Rear, Magazine, Head-jerk, Head-jerk/Hind, and Other) reliably increased from preCS to CS periods during conditioning, based on the assumption that these would be the behaviors most closely related to appetitive conditioning. Separate repeated-measures ANOVAs were calculated for each scored behavior with Period (preCS vs. CS) and Day (1-4) as factors. The main effect of Period was significant for all six behaviors, with significant increases from preCS to CS periods for Magazine ($F(1, 23) = 81.72$, $p < .001$, $\eta^2 = .78$), Head-jerk ($F(1, 23) = 86.01$, $p < .001$, $\eta^2 = .79$), and Head-jerk/Hind ($F(1, 23) = 29.80$, $p < .001$, $\eta^2 = .56$), behaviors. The other 3 behaviors all significantly decreased

in frequency from preCS to CS periods, Perambulate ($F(1, 23) = 14.92$, $p = .001$, $\eta^2 = .39$), Rear, ($F(1, 23) = 9.13$, $p = .006$, $\eta^2 = .28$), and Other ($F(1, 23) = 134.33$, $p < .001$, $\eta^2 = .85$.

Head-jerk and Head-jerk/Hind behaviors occurred relatively infrequently and were often difficult to distinguish, but appeared to change in similar ways. For this reason, the data from the 2 categories were combined into a new category named *Headjerk*. A repeated-measures ANOVA on the combined category showed that it too increased in frequency from preCS to CS periods during conditioning, $F(1, 23) = 92.23$, $p < .001$, $\eta^2 = .80$.

Graphs illustrating overall changes in responding from preCS to CS periods for each behavior (Magazine, Headjerk, Perambulate, Rear and Other) are shown in Figure 2. In the initial analysis, responses to the two CSs were combined. The same pattern of significant increases and decreases for each behavior was obtained when data for responding to the clicker and white noise stimuli were analyzed separately.

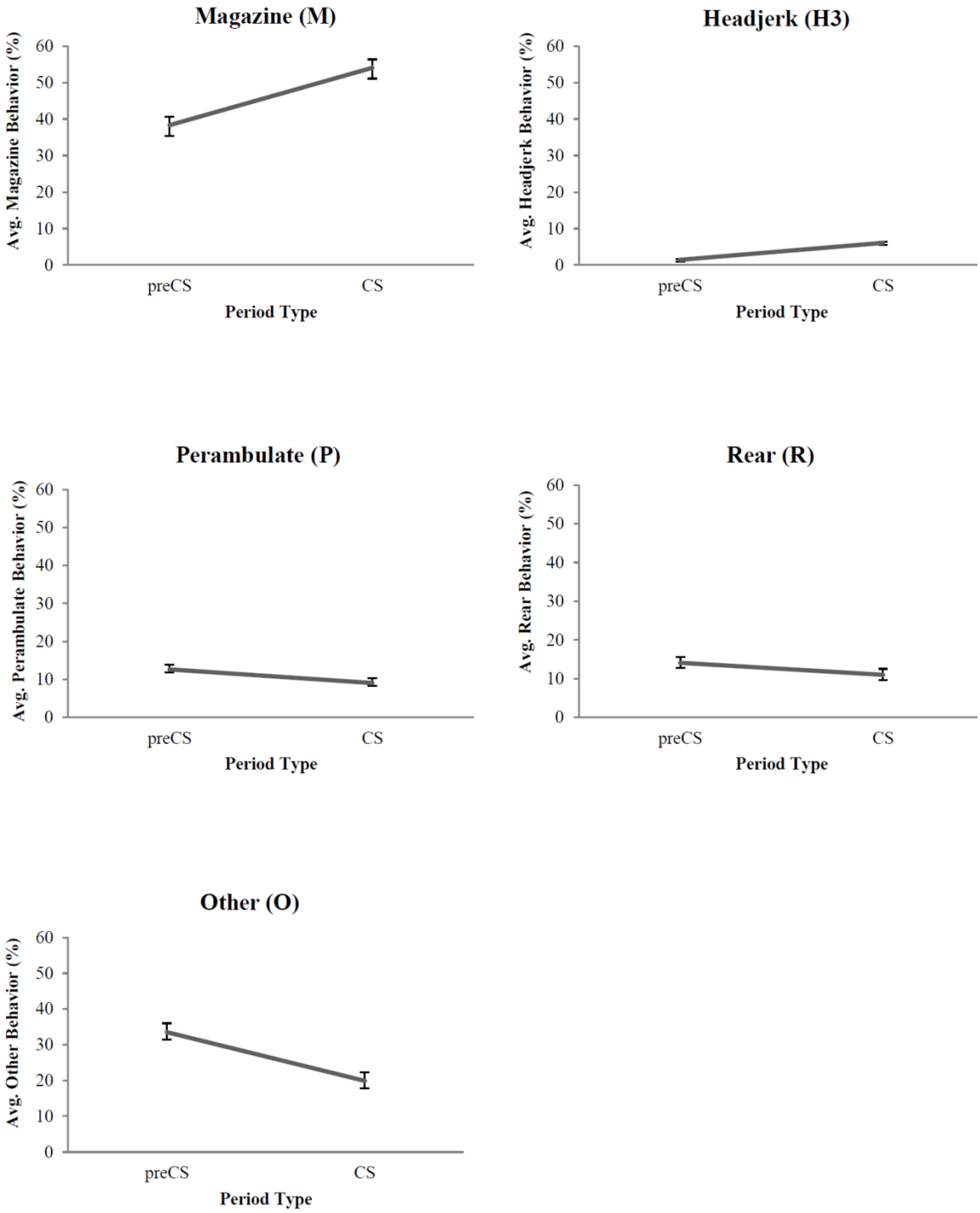
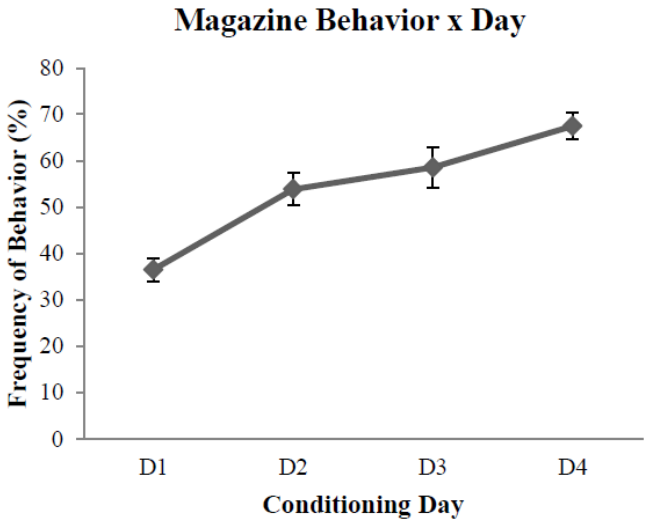


Figure 2: Mean frequency of responding during preCS and CS periods for each behavior. Error bars represent ± 1 standard error of the mean (SEM). ANOVA showed that Magazine and Headjerk behaviors increased significantly from preCS to CS periods, whereas Perambulate, Rear and Other significantly decreased from preCS to CS periods.

Changes across Days

Figure 3 shows how the frequency of Magazine and Headjerk behaviors changed across the days of conditioning. Magazine behaviors increased in frequency across conditioning, whereas Headjerk behaviors initially increased and then decreased across days. Results showed there was significant main effect of Day for both behaviors; therefore, repeated-measures ANOVAs were calculated on each pair of days to assess how Magazine and Headjerk behaviors changed across days. For Magazine behavior, responding significantly increased from Day 1 to Day 2, and from Day 3 to Day 4, $F(1, 23) = 66.05$, $p = <.001$, $\eta^2 = .74$, and 9.71, $p = .005$, $\eta^2 = .30$ respectively, but did not change from Day 2 to Day 3 $F(1, 23) = 2.66$, $p = .116$, $\eta^2 = .10$. For Headjerk behavior, on the other hand, there was a significant decrease in Headjerk behavior from Day 2 to Day 3, $F(1, 23) = 5.45$, $p = .029$, $\eta^2 = .19$ and no significant changes from Day 1 to Day 2, $F(1, 23) = .67$, $p = 4.23$, $\eta^2 = .03$, or Day 3 to Day 4, $F(1, 23) = .18$, $p = .672$, $\eta^2 = .01$. Overall, Magazine behavior increased across days while Headjerk behavior initially increased but then decreased. Given that the primary data were the percentage of observations on which a behavior occurred, any increase in one behavior must be matched by a decrease in one or more of the other behaviors. The pattern observed here suggests that Magazine behavior may be the behavior most closely related to conditioning in the present study.



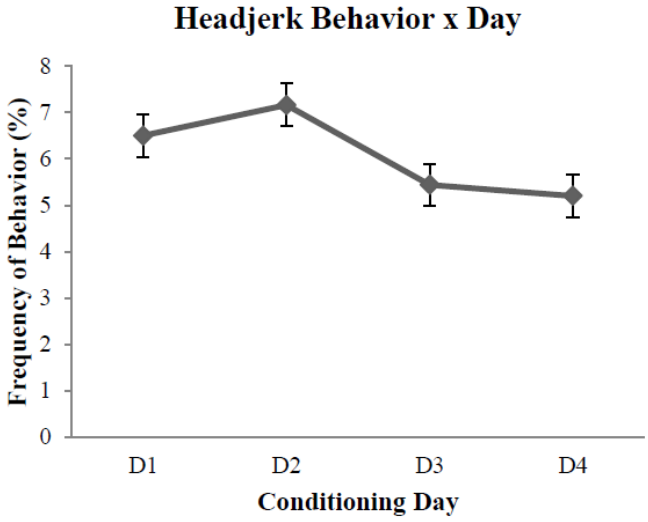


Figure 3: Mean scores and SEMs of Magazine and Headjerk behaviors across days of conditioning. Magazine behavior significantly increased from Day 1 to Day 2 and Day 3 to Day 4. Headjerk behavior significantly decreased from Day 2 to Day 3. Overall, Magazine behavior increased across days, while Headjerk behavior decreased.

Effects of Stimulus Preexposure and Drug Treatment on Conditioning

Separate mixed-model ANOVAs for Magazine and Headjerk behaviors were calculated to assess the effects of Stimulus Preexposure (clicker vs. white noise; non-repeated), Drug Treatment (Vehicle vs. Low vs. High; non-repeated), Conditioned Stimulus (clicker vs. white noise; repeated) and Day (1-4; repeated) on conditioning.

Magazine Behavior

Figure 4 illustrates the relative frequency of Magazine responses to both the clicker and white noise CSs as a function of Preexposure and Drug Treatment across Days of conditioning. Inspection of this figure suggested that the effects of Stimulus Preexposure on Magazine behavior depended on the Conditioned Stimulus, and that the effects varied with Drug Treatment. Results of the mixed-model ANOVA confirmed the existence of a significant main effect of Day, $F(3, 54) = 42.56$, $p < .001$, $\eta^2 = .70$, a significant Stimulus x Preexposure interaction, $F(1, 18) = 11.18$, $p = .004$, $\eta^2 = .38$, and a significant Stimulus x Drug interaction, $F(2, 18) = 14.59$, $p < .001$, $\eta^2 = .62$. None of the main effects for Stimulus, Preexposure or Drug Treatment were significant ($F(1, 18) = 2.10$, $p = .165$, $\eta^2 = .10$; $F(1, 18) = 2.19$, $p = .156$, $\eta^2 = .12$; $F(2, 18) = .362$, $p = .701$, $\eta^2 = .04$, respectively). Other interactions involving Stimulus Type approached, but did not reach conventional levels of significance, Stimulus x Drug x Day, $F(6, 18) = 2.23$, $p = .060$, $\eta^2 = .20$, and Stimulus x Day, $F(3, 54) = 2.23$, $p = .102$, $\eta^2 = .11$. No other or interactions were significant (the largest *F*-value was 2.19 with a *p*-value of .156).

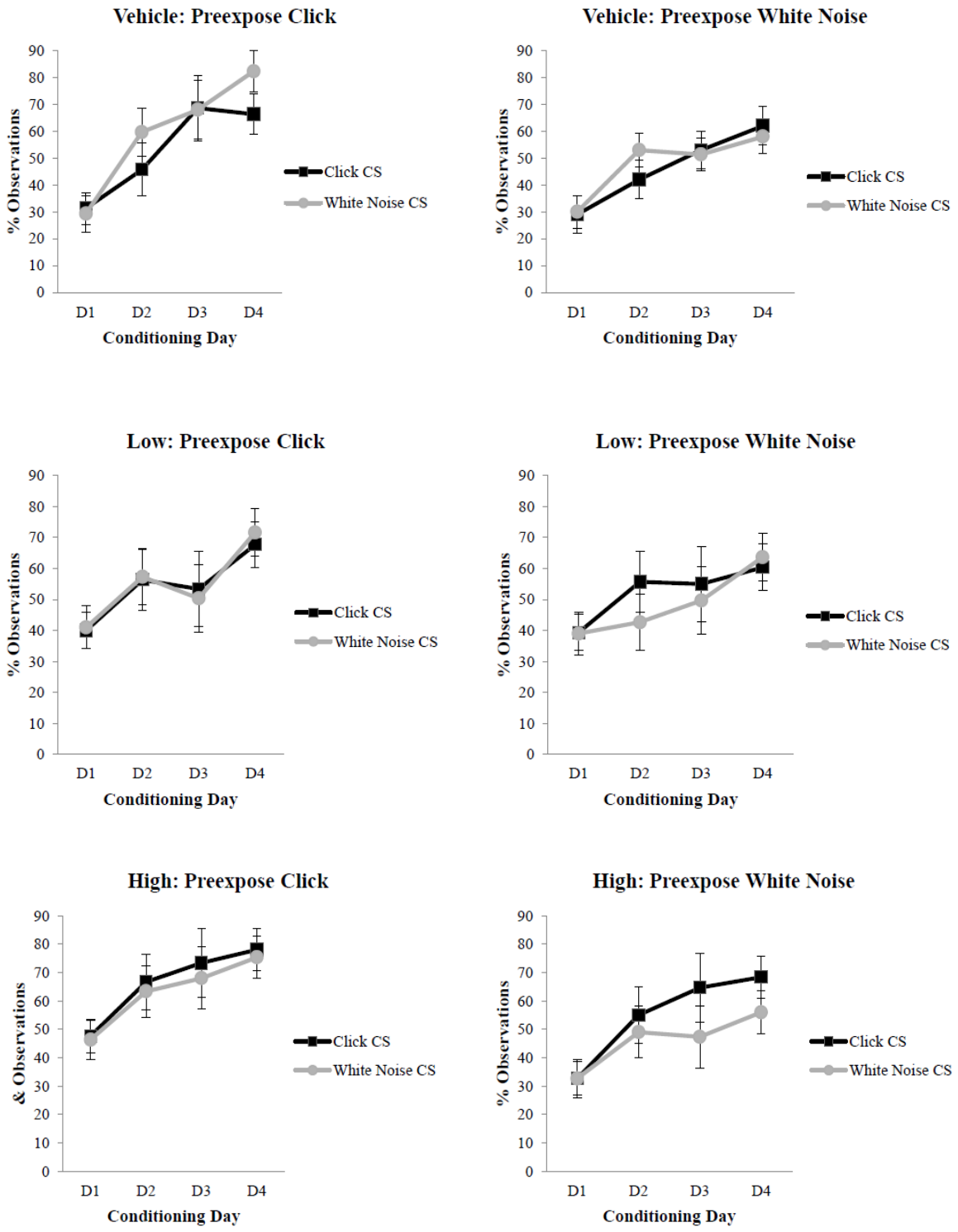


Figure 4: Effects of Stimulus Preexposure and Drug Treatment on Magazine responses to each Conditioned Stimulus across Days. Means and SEMs are reported.

Preexposure and Effects of Conditioned Stimulus Type. Figure 5 shows the frequency of Magazine behavior to the 2 conditioned stimuli as a function of which stimulus was initially exposed. Separate one-way repeated-measures ANOVAs were calculated to compare the frequency of Magazine responses to the clicker and white noise CSs, based on which stimulus was preexposed. These analyses showed that rats preexposed to the white noise responded significantly more to the novel clicker than to the white noise $F(1,11) = 4.93$, $p = .048$, $\eta^2 = .31$. On the other hand, when preexposed to the clicker, there was no difference in levels of responding to the two conditioned stimuli, $F(1, 11) = .91$, $p = .360$, $\eta^2 = .08$. Therefore, preexposure to the white noise produced latent inhibition for that stimulus, but preexposure to the clicker did not.

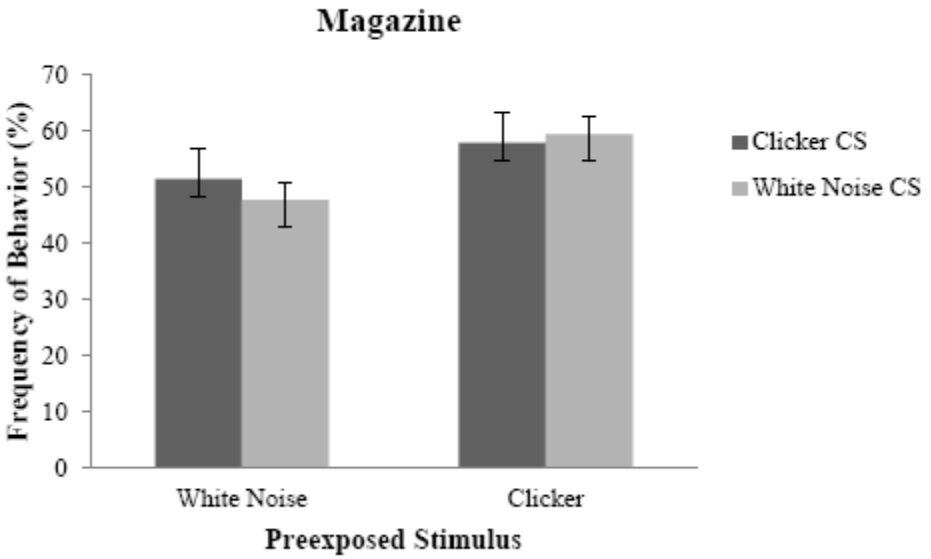


Figure 5: Magazine responses to each Conditioned Stimulus based on Preexposure. Means and SEMs illustrate that there was more responding to the clicker CS when preexposed to the white noise. Increased responding to the clicker when preexposed to the white noise symbolized a latent inhibition effect.

Drug Treatment. There was no main effect of Drug Treatment in the original mixed-model ANOVA but there was a significant Stimulus x Drug interaction. Figure 6 shows the relevant means for the interaction, and suggests that the stimulus that was a more effective CS varied with drug treatment. Rats in the Vehicle condition showed more Magazine behavior to the white noise than to the clicker, whereas the reverse was true for rats in the High dose condition. Rats in the Low dose condition appeared to respond at equal rates to the 2 stimuli. Separate one-way repeated-measures ANOVAs on Magazine responses calculated for each drug group confirmed these impressions of the data. Rats in the Vehicle condition responded significantly more to the white noise than to the clicker $F(1, 7) = 5.72$, $p = .048$, $\eta^2 = .45$, whereas rats in the High dose condition responded significantly more to the clicker than to the white noise, $F(1, 7) = 12.73$, $p = .009$, $\eta^2 = .65$. Rats in Low dose group showed comparable rates of responding to the two stimuli. $F(1, 7) = 1.48$, $p = .263$, $\eta^2 = .18$. One way to interpret this interaction would be to assume that the clicker was salient enough to provoke a fear reaction which retarded its conditioning in the Vehicle group, and that MK-801 decreased the aversive properties of the clicker which allowed it to condition more readily based on its salience. This idea will be further explored in the discussion.

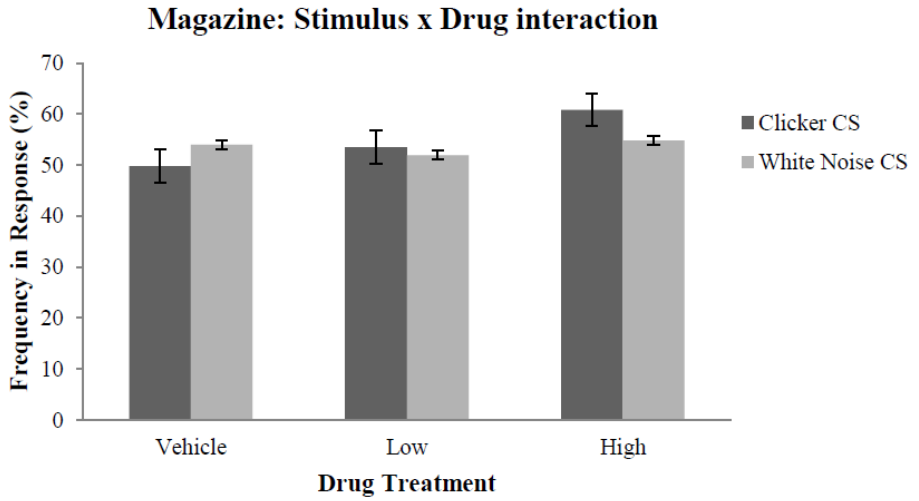


Figure 6: Differences in the rate of Magazine behavior when responding to each Conditioned Stimulus moderated by Drug Treatment. Means and SEMs of Magazine behavior show that the Vehicle group responded more to the white noise CS, the High dose group responded more to the clicker CS, and the Low dose group showed no discrimination between the 2 stimuli.

Headjerk Behavior

Figure 7 shows the effects of Stimulus Preexposure and Drug on Headjerk responses to each CS during conditioning. It appeared that there was more overall responding to the clicker than to the white noise, and that preexposure to the white noise enhanced this effect regardless of Drug Treatment. The mixed-model ANOVA confirmed that similar to Magazine behavior, there was a significant Stimulus x Preexposure interaction, $F(1, 18) = 8.88$, $p = .008$, $\eta^2 = .33$, and a significant main effect of Day, $F(3, 54) = 3.13$, $p = .033$, $\eta^2 = .15$, with behavior initially increasing and subsequently decreasing across days (seen earlier in Figure 3). Unlike Magazine behavior, there was a significant main effect of Stimulus, $F(1, 18) = 45.22$, $p < .001$, $\eta^2 = .72$. No other main effects or interactions were significant (the largest *F*-value was 1.53 with a *p*-value of -.218).

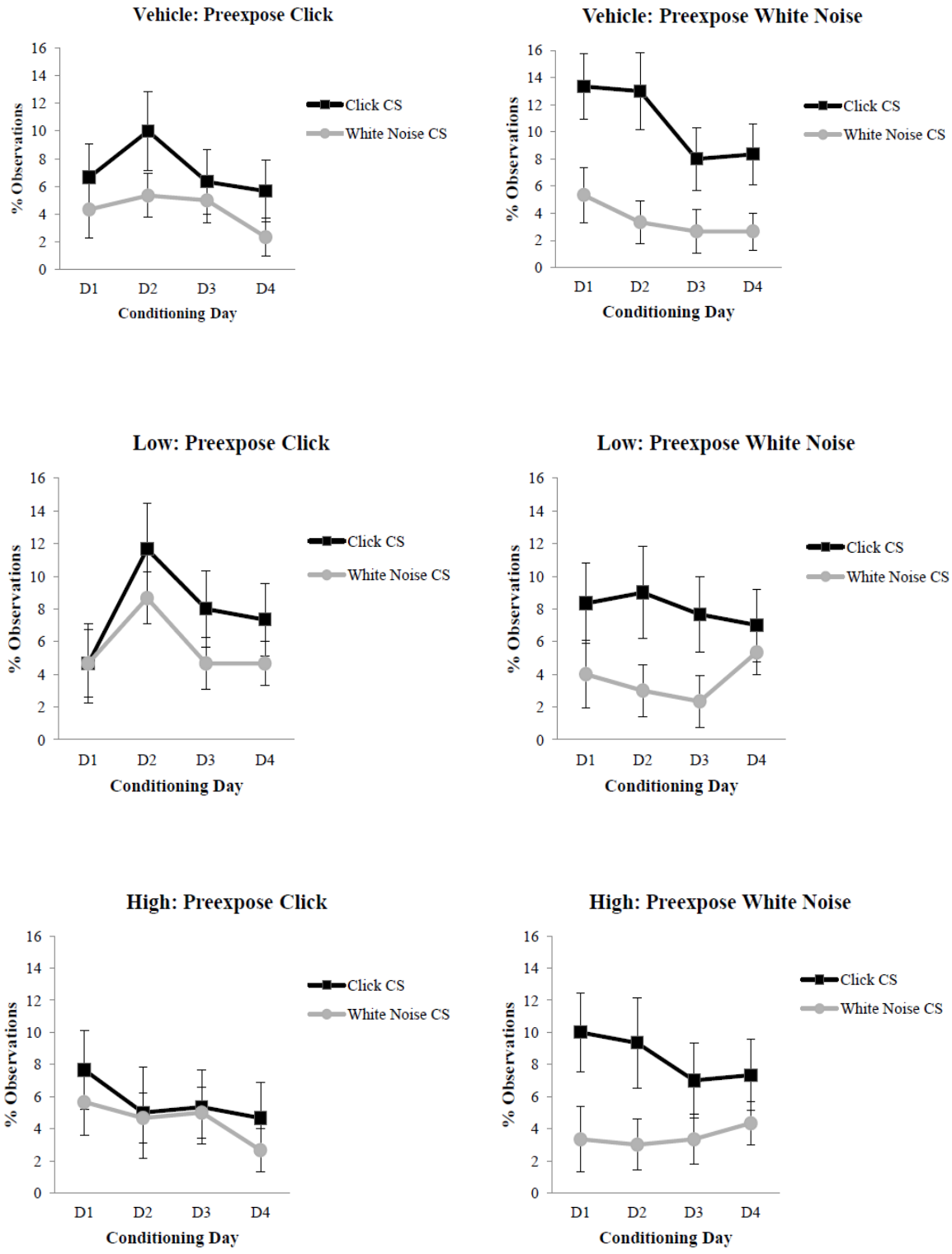


Figure 7: Effects of Stimulus Preexposure and Drug Treatment on Headjerk responses to each Conditioned Stimulus across Days. Means and SEMs are reported.

Preexposure and Effects of Conditioned Stimulus Type. Similar to Magazine behavior, there was a significant Stimulus x Preexposure interaction for Headjerk behavior. Figure 8 illustrates that when preexposed to the white noise, rats showed significantly more Headjerk behavior when responding to the clicker, $F(1,11) = 51.49$, $p < .001$, $\eta^2 = .82$. When preexposed to the clicker, however, rats again showed significantly more Headjerk responses to the clicker, $F(1, 11) = 6.94$, $p = .023$, $\eta^2 = .39$. A significant increase in the rate of responding to the novel clicker when preexposed to the white noise signified a latent inhibition effect. Conversely, when preexposed to the clicker, there was an enhancement of conditioning to the familiar clicker. Compared to the white noise, the clicker seemed to be a more effective CS. Regardless of Stimulus Preexposure, Drug Treatment, and Day of conditioning, the clicker CS evoked considerably more conditioned responding than the white noise CS which explained why there was a significant main effect of Stimulus, $F(1, 23) = 34.61$, $p < .001$, $\eta^2 = .60$.

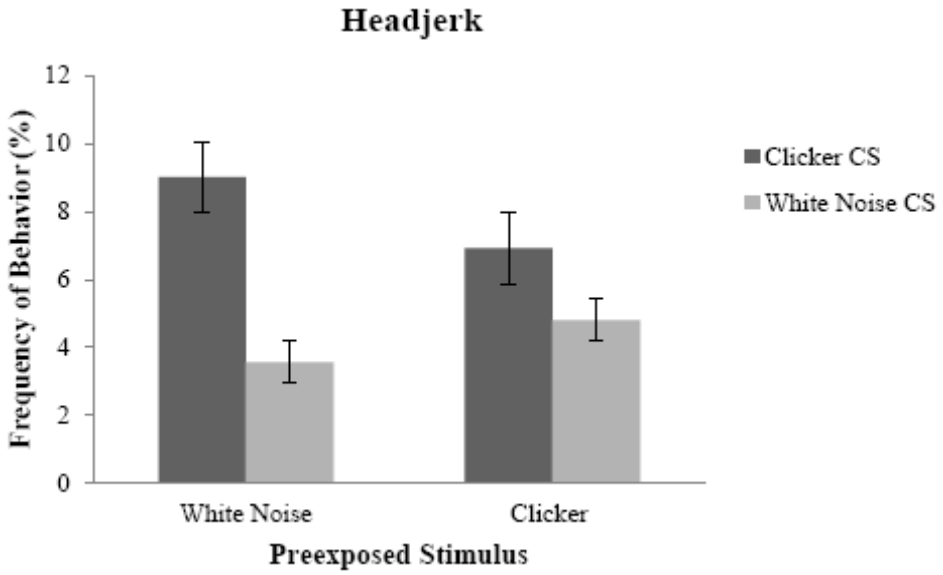


Figure 8: Headjerk responses to each Conditioned Stimulus based on Preexposure. Means and SEMs illustrate that there was more responding to the clicker CS than to the white noise CS independent of Preexposure. Increased responding to the clicker when preexposed to the white noise indicated a latent inhibition effect.

Drug Treatment. Unlike Magazine behavior, there were no significant effects of Drug Treatment found with Headjerk behavior. Therefore, MK-801 did not disrupt the latent inhibition effect found when rats were preexposed to the white noise.

Supplemental Analysis

Previous results indicated that Magazine behavior appeared to be the behavior that most closely represented conditioning and that the clicker and the white noise were not equally effective. Therefore, an additional mixed-model ANOVA was calculated for Magazine behavior that did not take CS type into account (Preexposure (novel vs. familiar; non-repeated; Drug Treatment (Vehicle vs. Low vs. High; non-repeated; and Day (1-4; repeated). Similar to the previous analyses, there was a significant main effect of Day, $F(3, 42) = 42.89$, $p < .001$, $\eta^2 = 1$, and there was no main effect of Drug Treatment, $F(2, 42) = .40$, $p = .676$, $\eta^2 = .10$. However, unlike the previous analyses, there was a main effect of Preexposure, $F(1, 42) = 4.9$, $p = .04$, $\eta^2 = .556$, with rats responding significantly more to the novel stimulus. Ultimately, there was more conditioned responding when a stimulus was novel than when a stimulus was familiar, regardless of whether the stimulus was the clicker or the white noise. This analysis strengthens the ability to claim that a latent inhibition effect is present in all Drug Treatment groups, despite the differences in the salience of the CSs.

CHAPTER 4. DISCUSSION

Magazine and Headjerk behaviors were identified as the behaviors that most closely represented appetitive conditioning due to their significant increase in relative frequency from preCS to CS periods. A change in one behavior had to be matched by a change in the other behavior; therefore, as Magazine behavior continued to increase

across days, Headjerk behavior correspondingly decreased. Conversely, Holland (1977) found that Magazine behavior decreased while Head-jerk and Head-jerk/Hind behaviors increased. In this study, the continual increase in Magazine behavior across days suggested that it was likely the behavior most associated with conditioning.

For both Magazine and Headjerk behaviors, when rats were preexposed to the white noise it created more rapid acquisition of conditioned responding to the novel clicker, revealing a latent inhibition effect. When the clicker was preexposed, making it familiar, there were no significant differences in Magazine responding to either CS; however, when looking at Headjerk behavior, the rats again conditioned more rapidly to the clicker CS. Essentially, the clicker was a more salient stimulus when compared to the white noise despite physically equating them at the start of the study. In the future, it would be advantageous to calibrate the clicker and white noise relays in each chamber prior to each day's sessions to ensure their physical intensity is constantly equivalent.

Independent of all other factors, the Vehicle rats showed significantly more Magazine behavior in response to the white noise CS, while those in the High dose group responded significantly more to the clicker CS. Although the Vehicle and High dose groups showed somewhat different responses to the white noise and the clicker, there was no evidence MK-801 disrupting latent inhibition. Additionally, there is no way of claiming whether MK-801 enhanced the latent inhibition effect or not. According to Gaisler-Solomon & Weiner (2003), if conditioning trials were implemented to the point where conditioning behavior was no longer evident in control animals, but still evident in drugged animals, only then it would be possible to make the claim of an enhanced latent inhibition. Unlike results found by Holland (1997), in this study, Magazine behavior

continued to increase, while Headjerk decreased, on the final day of conditioning for all animals regardless of Drug Treatment. Despite this small discrepancy, there was still conditioning occurring on the last day of testing for both control and drugged rats; therefore, in the future it would be beneficial to run additional conditioning trials until conditioning was no longer evident.

Based on the results concerning the interactions between Stimulus and Preexposure, and Stimulus and Drug Treatment, one could argue that the clicker contained unconditioned aversive properties. When the clicker was preexposed, making it familiar, it created a habituated fear response, whereas when the white noise was preexposed, making the clicker novel, its aversive properties interfered with conditioning to the white noise. In regards to Drug Treatment, the Vehicle rats responded significantly more to the white noise because the clicker elicited a fear reaction that retarded its conditioning ability. On the other hand, the anxiolytic properties of MK-801 reduced the aversive properties of the clicker allowing rats to condition more readily to it. This is just speculation; however, it would be valuable to ensure that both conditioned stimuli are constantly comparable throughout the study.

Another explanation for the differences in the salience of the CSs could be that the frequency of the clicker, 2 Hz, was not high enough. Perhaps the space between each individual click was too large making the CSs completely incomparable. It would be worthwhile to increase the Hz of the clicker so each individual click would be presented much closer together in order to resemble something more similar to a tone rather than a disjointed click. That way, the clicker would sound more constant, like the white noise, making the CSs more comparable. Another way to perhaps make the two stimuli more

comparable would be to interrupt the white noise to make it sound more fragmented like the clicker.

By and Large, all rats, regardless of Drug Treatment, displayed latent inhibition when preexposed to the white noise; however, there is speculation that this could be the result of the characteristics of the clicker CS. Additional analyses showed that despite the differences in the effectiveness of the CSs, there was still more conditioned responding to the novel rather than familiar stimulus. Since both control and drugged rats showed latent inhibition, despite significant differences in the CSs, it is possible that NMDA receptors may not be critical for the latent inhibition effect. There is not enough evidence to confirm an enhanced or a disrupted latent inhibition effect as a consequence on NMDA antagonism; therefore, conclusions regarding hippocampal involvement in latent inhibition remain uncertain, for that reason, it remains worthwhile to explore the context specificity of latent inhibition.

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APPENDIX 1

Drug x Chamber Assignment

Rat ID	Chamber 0A (1)	Chamber 0B (2)	Chamber 1A (3)
MA-G1-01	Vehicle	-	-
MA-G1-02	-	0.05 mg/kg	-
MA-G1-03	-	-	0.1 mg/kg
MA-G2-04	0.1 mg/kg	-	-
MA-G2-05	-	Vehicle	-
MA-G2-06	-	-	0.05 mg/kg
MA-G3-07	0.05 mg/kg	-	-
MA-G3-08	-	0.1 mg/kg	-
MA-G3-09	-	-	Vehicle
MA-G4-10	Vehicle	-	-
MA-G4-11	-	0.05 mg/kg	-
MA-G4-12	-	-	0.1 mg/kg
MA-G5-13	0.1 mg/kg	-	-
MA-G5-14	-	Vehicle	-
MA-G5-15	-	-	0.5 mg/kg
MA-G6-16	0.5 mg/kg	-	-
MA-G6-17	-	0.1 mg.kg	-
MA-G6-18	-	-	Vehicle
MA-G7-19	Vehicle	-	-
MA-G7-20	-	0.05 mg/kg	-
MA-G7-21	-	-	0.1 mg/kg
MA-G8-22	0.1 mg/kg	-	-
MA-G8-23	-	Vehicle	-
MA-G8-24	-	-	0.05 mg/kg
MA-G9-25	0.05 mg/kg	-	-
MA-G9-26	-	0.1 mg/kg	-
MA-G9-27	-	-	Vehicle