THE EFFECTS OF MK-801 ON TACTILE DISCRIMINATION LEARNING IN 28 DAY OLD RATS

by

Jenna Cohen

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Degree in Psychology with Distinction.

Spring 2007

THE EFFECTS OF MK-801 ON TACTILE

DISCRIMINATION LEARNING IN 28 DAY OLD RATS

by

Jenna Cohen

Approved: _____

Mark Stanton, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____

Anna Klintsova, Ph. D
Committee member from the Department of Psychology

Approved: _____

Christine Ohannessian, Ph. D
Committee member from the Board of Senior Thesis Readers

Approved: _____

Rhonda Hyde, Ph.D.
Chair of the University Committee on Student and Faculty Honors

ACKNOWLEDGMENTS

I would like to thank Dr. Stanton for allowing me the opportunity to accomplish this senior thesis, and for the guidance he has given me along the way. Thank you to Deborah Watson for being a mentor throughout the entire process. Thank you to Dr. Klintsova and Dr. Ohannessian for participating as committee members, and to Jenn Burr and Nicole Buzin for helping to run sessions.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

The present study examines the role of the NMDA receptors in a tactile discrimination reversal task in weanling rats. An NMDA antagonist, MK-801 (dizocipline maleate) was used to investigate the effects of NMDA antagonism on a form of non-spatial reversal learning. Subjects were 28-day-old rats that were trained in a T-maze to examine their performance on acquisition and reversal of a tactile discrimination task. Subjects received either a dose of 0.06 mg/kg of MK-801 or saline vehicle through an intraperitoneal injection prior to each testing session. In Experiment 1 all subjects received saline during three session of acquisition, and then half of the subjects received drug treatment and the other half received saline during five sessions of reversal. Sessions occurred two times a day, and at least six hours apart. The group that was treated with MK-801 was significantly slower to learn the task throughout reversal ($p< 0.002$). In Experiment 2 half of the subjects received drug treatment and the other half received saline during three sessions of acquisition in order to further examine the role of NMDA receptors in tactile discrimination. The group that was treated with MK-801 performed significantly worse during acquisition as well ($p<0.06$). Results suggest NMDA receptor as involved in acquisition and reversal of tactile discrimination. Furthermore, task difficulty, as well as different brain regions and mechanisms are involved in learning of this tactile discrimination reversal task.

Chapter 1

INTRODUCTION

Learning and memory are important and complex cognitive processes that are possessed by both humans and animals. N-methyl-D-aspartate (NMDA) receptors are one form of glutamate receptors and are involved in learning and memory. These NMDA receptors are found in the brain, with the highest concentrations in the frontal cortex, striatum and hippocampus, and have been found to be involved in learning and memory (Wong et al., 1986). Furthermore, it is known that long term potentiation (LTP) in the hippocampus, a form of synaptic plasticity arising from experience, is important for learning and memory. This is thought to be because NMDA receptors are involved in the LTP of neurons in the hippocampus (Bashir et al., 1994). NMDA receptor antagonists, such as the non-competitive NMDA receptor antagonist, MK-801 (dizocipline maleate), are used to study the role of NMDA receptors in animals. The examination of the role of the glutamatergic NMDA receptors is important because they have been linked to various neurological disorders. It has been suggested that glutamate dysfunction, plays a large role in the pathophysiology of both autism (Carlsson, 1998, Shinohe et al., 2006) and schizophrenia (Goff & Coyle et al., 2001), particularly hyperglutamatergic action (Carlsson, 1998). NMDA receptor antagonism in humans mimics many of the behavioral symptoms of individuals with autism (Carlsson, 1998) and in schizophrenic patients (Goff & Coyle et al., 2001). Therefore, animal models using NMDA receptor

antagonism may be useful in evaluating neurological processes involved in these disorders, and pharmacological treatments for them.

NMDA receptor antagonism results in impaired learning and memory on many spatial and non-spatial tasks in animals. Specifically, NMDA receptor antagonism impairs reversal learning in weanling and adult rats. Chadman et al. (2006) found that MK-801 impairs reversal of a spatial discrimination task in weanling rats. MK-801 also impairs reversal in adult mice in a water T-maze task (Bardgett et al., 2003). NMDA antagonists have also been found to effect non-spatial reversal tasks. MK-801 impairs the reversal of an odor discrimination task in weanling rats (Griesbach et al., 1998), and a single patterned alternation task in pre weanling rats (Highfield et al., 1996). Reversal learning is difficult because it requires that an animal inhibit formerly learned responses (Bouton, 1993). It is known that NMDA receptor antagonists impair spatial learning. However, their effect on non-spatial learning is not clear.

Several studies examine the processes involved in spatial and non-spatial learning in animals (Wishaw, 2004, Oliveira et al., 1997, Naghdi and Harooni, 2005, Winocur et al., 2006, and Murray et al., 1995). The present study examines the effect of an NMDA receptor antagonist, MK-801 (dizocipline maleate) on a non-spatial, tactile discrimination reversal task in weanling rats. MK-801 binding with NMDA receptors peaks in the rat neostriatum at postnatal day 28 (Colwell et al., 1998). Therefore, the rats in the present study are postnatal day 28 on the first day of testing. A study by Chadman et al. (2006) found that MK-801 impairs reversal, but not acquisition on a spatial discrimination task, showing that NMDA receptors are involved in learning reversal of a spatial task.

The present study asked whether the results of Chadman et al. (2006) are specific to spatial reversal or if they can be generalized to non-spatial discrimination tasks as well, specifically tactile reversal discrimination learning. Experiment 1 examines the effect of MK-801 on reversal of a tactile discrimination task. A dose of 0.06 mg/kg of MK-801 significantly impaired reversal performance of subjects, compared with controls. Experiment 2 examines the effect of MK-801 during acquisition of the same tactile discrimination task. MK-801 was found to also impair acquisition of the task.

Chapter 2

EXPERIMENT 1: METHOD

Subjects

Subjects were 30 Long-Evans rats (16 female, 14 male) that were the offspring of 18 time-bred females obtained from Harlan Laboratory Breeders (Fredrick, Maryland). Dams were housed in clear polypropylene cages, in a facility maintained on a 12:12 hour light/dark cycle beginning at 700 hr. Dams were left undisturbed with water and rat chow except for routine cage changes and periodic checks for births during the light cycle. The day of birth was designated PND 0 (postnatal day). Litters were then culled to 8 pups, 4 males and 4 females (when possible) on PND 3.

The pups were weaned from their mothers on PND 21 and housed with same sex littermates with a continuous supply of food and water until the beginning of experimentation. All pups were deprived of food and water on PND 26, except for one that was deprived on PND 25. Of the 30 pups initially included, 2 females were excluded because a complete set of data failed to be collected. One ceased to run after the first session, the other one failed to run during session eight. Of the 28 pups included, 27 of the pups were 28 on the first day of testing, and one of the pups was 27 on the first day of testing. This age difference is negligible, as previous studies have shown that one day of age doesn't influence learning on T-maze tasks. Empirical

analysis also showed no statistical difference in performance between day-older or day-younger subjects.

Apparatus

Four identical T-mazes, scaled for use with weanling rats, were used to train subjects. The T-maze design and procedure are detailed in Freeman & Stanton (1991). Two T-mazes were set on one tabletop about 1.5 feet apart, allowing them to be operated at the same time by a single experimenter. The T-maze was made of clear Plexiglas covered with brown paper on all sides except for the hinged doors on top of each of three arms. Each T-maze consisted of a left and right goal box arm, and a start arm perpendicular to the goal box arms, all of which were the same size. At the end of each goal box arm was a small metal cup that catches light cream (commercially available half-and-half) pumped from a syringe when a correct choice was made. The cream was contained in a 20 mL syringe and was connected to the metal cup by PE-160 tubing. For correct choices the syringe pump dispensed .07 mL of light cream. Separating the start arm and the goal box arms from each other were three computer-controlled, pneumatically operated doors, which created a choice point for the pup. A 25-W amber bulb found directly above the choice point illuminated that area. A computer recorded correct and incorrect responses, measured when the subject broke a photoelectric beam near the end of a goal box. The computer also recorded latencies for the pup to break the photoelectric beam, controlled the pneumatic doors, and the syringes that pumped light cream into the metal cups. In between trials, each subject was kept in a clear Plexiglas intertrial interval (ITI) box with hinged, ventilated lids and paper towels coating the bottom.

The tactile difference was created by adding coarse wire mesh (1/4 in. mesh commercially available) to the floor of the T-maze. A strip of mesh half the width of the start arm was laid on the side of the start arm designated to have the positive tactile cue. A strip of mesh fitted to the entire size of the goal arm was placed in the corresponding goal arm designated to have the positive tactile cue. The experimenter periodically moved the mesh from side to side on a predetermined quasi-random order, with no pup going the same direction with the same tactile cue more then three trials in a row (Fellows, 1967). This was done in order to eliminate the possibility of any spatial cues being associated with the reward.

Design

The design involved between-subject variables of treatment (0.06 mg/kg MK-801 or .09% saline vehicle), and tactile cue (designated to follow mesh path or follow smooth path). These conditions of treatment and tactile cue were counterbalanced against maze, sex, and dam. Subjects were trained to follow one of the two tactile cues during 3 sessions of acquisition and the opposite tactile cue during 5 sessions of reversal. Each session consisted of four blocks of 12 trials each, totaling 48 trials for each subject in each session. Subjects received 144 trials in acquisition, and 240 trials in reversal, totaling 384 trials for each subject. Trials where a subject did not run in the allotted three minutes were removed from the data set and new averages were calculated. Two sessions occurred each day, for four consecutive days. Sessions lasted about one and a half hours each, and took place six hours apart. For acquisition the design was a 2 (Treatment) by 3 (Session) by 4 (Block) mixed factorial design.

For reversal the design was a 2 (Treatment) by 5 (Session) by 4 (Block) mixed factorial design.

Deprivation and Acclimation

On PND 26, at approximately 1600 hr, subjects were deprived of food, water, and social contact. Pups were weighed, had their tails marked for identification, and were hand fed a drop of light cream directly into their mouths by way of a 10-mL syringe. They were then placed into individual opaque Plexiglas cages where a tablespoon was fitted onto the side of the cage to hold light cream for post feeding. Upon deprivation these spoons were filled with 1.0 mL of light cream intended to reduce taste neophobia and facilitate consumption of the reward during the experiment (Freeman & Stanton, 1991).

During acclimation, subjects were trained in groups of four to six, with two or three rats running on one maze. Acclimation consisted of three training sessions on PND 27, each approximately four hours apart. There were two goal box training (GBT) sessions, and a forced run (FR) intended to teach subjects to run and consume the light cream reward at the end of the goal box. During the first GBT, pups were placed into one goal box arm until they consumed the light cream reward, or until 3 minutes had passed. If they had not consumed the reward within three minutes, they were hand fed 0.7 mL of the light cream. They received 6 consecutive trials in the same arm. During the second GBT, pups were placed in the opposite goal box arm until they consumed the light cream reward for another 6 trials. Mesh was placed over the floor of both goal arms on one maze, and the other maze remained smooth, thus pre-exposing half of the subjects to each tactile cue. The forced run consisted of 12

trials, 6 to each arm in an irregular schedule. Mesh was placed in each maze over half of the start arm and the corresponding goal arm. The subject was placed in the start arm and only one of the goal box arms was opened. Mesh was placed on different sides of the maze so that each pup was exposed to the both tactile cues on each side of the T-maze an equal number of times. The subjects were rewarded with 0.7 mL of light cream after breaking the photocell at the end of the goal arm. They were fed milk at the end of these sessions to retain 85% of their weight at the time of deprivation.

Acquisition and Reversal

All subjects were assigned to one of four experimental conditions, MK-801 treatment following mesh (n = 6), Saline treatment following mesh (n = 8), MK-801 treatment following smooth (n = 8), Saline treatment following smooth (n = 6). Subjects were trained in the same squads they were acclimated in. Training took place over eight sessions (two sessions per day) and four days. There were two acquisition sessions on Day 1. The morning session on Day 2 was the last session of acquisition and the afternoon session on Day 2 was the first session of reversal. Two sessions of reversal took place on Day 3 and the last two sessions of reversal took place on Day 4. Prior to each session, pups were weighed and given intraperitoneal injection with either 0.06 mg/kg MK-801 or saline in a 1.0 ml/kg volume. Pups were then placed in ITI boxes for 30 minutes prior to the start of each session, to allow the drug to reach its full effect.

During the three sessions of acquisition, all pups were given an injection of saline; the MK-801 treatment was not introduced until reversal. During acquisition, mesh was placed by the experimenter on different sides of the maze according to a

balanced, but irregular sequence (Fellows, 1967) throughout a session of 48 trials. Subjects were placed in the start arm and the experimenter pressed a button, which caused the computer to initiate the trial. The start arm door opened, followed two seconds later by the opening of both goal arm doors, revealing both tactile cues and creating a choice for the subject. Reward of 0.7 mL of light cream was contingent upon choosing the correct arm and breaking the photocell at the end of the arm. The maze arms were lowered at the end of each trial after a choice was made and the pups remained in the goal box for a period of 20 seconds and then were returned to their ITI boxes. They waited in the ITI box until the other subjects in the squad completed their trials (typically 60-90 seconds), before being given their next trial. After each morning session, pups were given an amount of light cream needed to maintain 85% of the body weight at deprivation. After each afternoon session ended at approximately 1800 hr, pups were given unlimited access to food and water until deprivation of water resumed at 2200 hr. This deprivation schedule allows pups to gain weight normally while remaining well motivated during the experiment (Watson et al., 2006).

The five sessions of reversal were the same as the sessions of acquisition except for the following differences. A dose of 0.06 mg/kg of MK-801 was given to half of the subjects and the other half were given an injection of saline. A dose of 0.06 mg/kg was used because it has previously been found to effect reversal but not acquisition in spatial position habitat tasks (Chadman et al, 2006). In reversal, subjects had to learn to follow the opposite tactile cue from the one learned in acquisition. Reward was contingent upon choosing the arm with the tactile cue that had not been rewarded in acquisition (i.e., if in acquisition the rewarded cue was mesh, in reversal,

the rewarded cue was smooth). In all other respects, the procedure during reversal was as just described for acquisition.

Chapter 3

EXPERIMENT 1: RESTULTS

Preliminary analysis showed that there were no significant effects for maze (1, 2, 3, or 4), sex, or tactile cue (mesh or smooth). Data were therefore pooled across these factors and then subjected to analysis of variance (ANOVA) performed separately on acquisition versus reversal. A 2 (treatment) x 3 (session) x 4 (block) ANOVA was performed for acquisition sessions and a 2 (treatment) x 5 (sessions) x 4 (block) ANOVA was performed for reversal sessions.

The ANOVA of acquisition sessions revealed main effects for sessions ($F(2,52) = 69.75$, $p<0.001$), and blocks ($F(3,78) = 26.12$, $p<0.001$), but not for

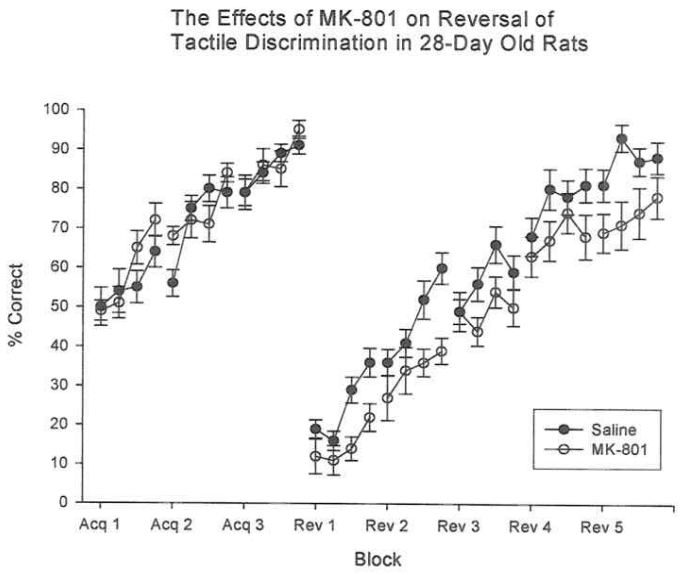

Figure 1. The effects of the NMDA receptor antagonist, MK-801 on reversal of a tactile discrimination task in 28-day-old rats.

treatment ($F < 1$). Data from the three sessions of acquisition can be found in Figure 1. The lack of main effect for treatment is consistent with the fact that this factor was a "dummy variable" since subjects were all given saline treatment and none of the pups received MK-801. No other statistical effects were significant, except for an interaction of treatment x sessions x blocks ($F (6, 156) = 2.34$, $p< 0.05$). This effect was unexpected because treatment was a dummy variable and each group received saline. Newman-Keuls test showed that this significant interaction existed in block 1 of session 2, where the group assigned to get MK-801 treatment during reversal had a higher percentage correct then the group that was assigned to get saline treatment during reversal. However, at the end of acquisition, each group was performing at the same level. Therefore, each group began the reversal sessions at the same performance level following acquisition of the tactile discrimination.

A 2 (treatment) x 5 (session) x 4 (block) ANOVA was performed for reversal sessions. No main or interaction effects were found for sex or maze, so data was pooled across these factors. For cue, a significant interaction with treatment was found ($F (1,24) = 5.009$, $p < .035$) that resulted from a small tendency for treatment effects to be larger in the rats for which mesh was the rewarded cue during acquisition and smooth was the rewarded cue during reversal. This effect, though significant, was numerically small and didn't alter conclusions about the primary treatment effects in this study, so data was pooled across this factor also. Data for the five sessions of reversal can be found in Figure 1. Results showed that there was a main effect for treatment, ($F (1, 26) = 11.82$, $p< 0.002$). As expected, subjects that received MK-801 prior to reversal sessions performed at a level lower then the control group. There were no interaction effects between treatment and session or block suggesting that this

impairment in performance remained constant across reversal training. The ANOVA of reversal sessions also revealed main effects for sessions ($F(4,104) = 138.35$, $p<0.001$), and blocks ($F(3,78) = 17.46$, $p<0.001$). No other effects were found to be statistically significant except for an interaction of sessions x blocks, ($F(12, 312) = 2.81$, $p< 0.002$). Subject's performance increased across blocks and sessions beginning at a percent correct well below chance, due to interference from the previously learned acquisition of the tactile discrimination task. Over the five sessions of reversal both the MK-801 group and saline group were able to learn the reversal of a tactile discrimination. However, throughout reversal the saline group achieved a higher percentage correct throughout reversal sessions.

Chapter 4

EXPERIMENT 2: METHOD

The methods used in this experiment were the same as the methods used in Experiment 1, except where noted.

Subjects

Subjects were 24 Long-Evans rats (13 female, 11 male) that were the offspring of 10 time-bred females obtained from Harlan Laboratory Breeders (Fredrick, Maryland). The pups were reared, weaned, and housed as previously described. All pups were deprived of food and water on PND 26, except for four that were deprived on PND 25. Of the 26 pups initially included, 1 female was excluded because a complete set of data failed to be collected due to unexpected death during the days of experimentation. Of the 25 pups included, 21 of the pups were 28 on the first day of testing, four of the pups were 27 at first day of testing.

Apparatus

The T-maze apparatus were the same ones used in Experiment 1 (except only one set of two mazes was used).

Design

The design involved the same between-subject variables of treatment and tactile cue. Subjects were trained to follow one of the two tactile cues during three

sessions of acquisition and no sessions of reversal took place. Two sessions occurred each day, for one and a half days. For acquisition the design was a 2 (Treatment) by 2 (Cue) by 3 (Session) by 4 (Block) mixed factorial design.

Procedure

All subjects were assigned to one of four experimental conditions, MK-801 treatment following mesh (n =6), Saline treatment following mesh (n =6), MK-801 treatment following smooth (n =6), Saline treatment following smooth (n =5). The procedure for deprivation, maze acclimation, and training were the same as in Experiment 1, except that subjects received doses of either MK-801 and saline vehicle during acquisition. There were no sessions of reversal.

Chapter 5

EXPERIMENT 2: RESULTS

Results showed that there were no significant main effects for maze, sex, or tactile cue, therefore data was pooled across these factors. There was an interaction effect for session x block x tactile cue, ($F(6, 126) = 2.33$, $p < 0.05$). Pups rewarded for following mesh learned slightly faster than pups rewarded for following smooth. However, because this effect had no interaction with treatment, data was pooled across tactile cue and a 2 (treatment) x 3 (session) x 4 (block) ANOVA was performed. Data from the acquisition sessions can be found in Figure 2. Results show that during three

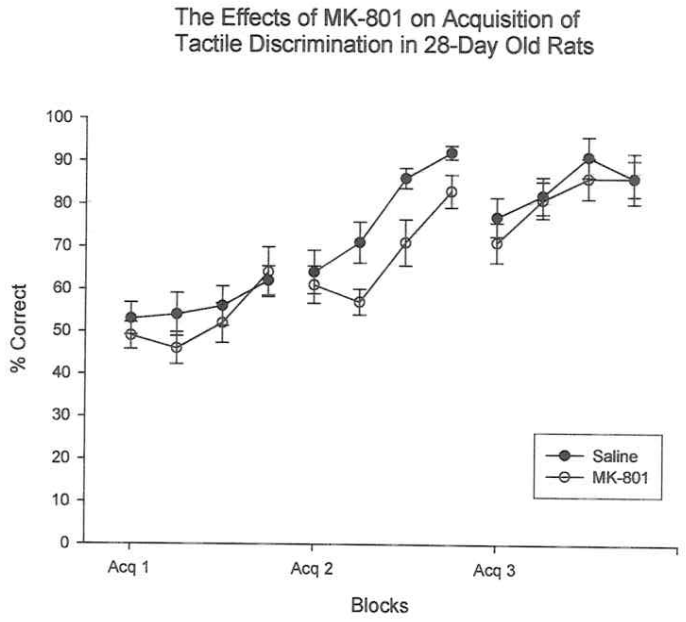

Figure 2. The effects of the NMDA receptor antagonist, MK-801 on acquisition of a tactile discrimination task in 28-day-old rats.

sessions of acquisition, performance was different between the group that received MK-801 and the group that received saline. Treatment yielded a significant main effect ($F(1, 21) = 3.98$, $p < 0.06$). This effect of drug treatment on acquisition was unexpected and showed that a dose of 0.06 mg/mL of MK-801 does impair performance on acquisition of tactile discrimination. Post hoc analysis using Newman Keuls revealed that the treatment effect occurs in block 2 and block 3 of session 2.

Both groups showed an increase in percent correct choice over the three sessions, beginning at chance and increasing to about 90% correct, demonstrating that they did learn the acquisition of tactile discrimination. There was a significant main effect of session, ($F(2,42) = 65.80$, $p < 0.001$), and blocks, ($F(3,63) = 24.24$, $p < 0.001$). There was also a significant interaction between session x block, ($F(6,126) = 3.23$, $p < 0.006$). There is an increase in percent correct choice across blocks and across sessions regardless of treatment group, however the group that received MK-801 performed at a lower level during the three sessions of acquisition.

Chapter 7

ANALYSIS OF ERRORS

There are different types of errors made when learning a task that may be controlled by different brain regions or different neuropharmacological mechanisms (Palencia & Ragozzino, 2004). Trials to criterion is a measure of learning speed. It is reached when a subject makes at least 10 correct choices in a block of 12 consecutive trials. If a subject did not achieve this throughout testing, they were assigned the maximum number of trials (144 in acquisition, 240 in reversal). Perseverative errors occur when a subject makes an incorrect choice three or more times in a block of four consecutive trials, therefore measuring the ability of the subject to shift away from the previously learned response. In contrast, any error that is not a perseverative error is a regressive error, which measures the ability of the subject to learn the new desired response, once they are no longer perseverating on the old response. Total errors are the sum of all perseverative and regressive errors that a subject makes (Palencia & Ragozzino, 2004).

A one-way ANOVA of reversal was conducted for trials to criterion, perseverative, regressive, and total errors to examine the types of errors made by subjects during tactile discrimination reversal. Then error data from both acquisition and reversal was compared with data from Chadman et al. (2006) to investigate the effect of task difficulty and learning ability in spatial versus non-spatial tasks on sensitivity to MK-801. A factorial ANOVA was conducted to compare this data for each type of error. Comparison data from Chadman et al. (2006) consisted of 19

subjects, PND 30 on the day of testing. Half were given saline and the other half were given the same 0.06 mg/kg dose of MK-801 used in this study.

Analysis of Reversal Errors in Experiment 1

For reversal sessions in the present study, a one-way ANOVA revealed no main effects for trials to criterion or regressive errors. There was a main effect of treatment for perseverative errors ($F(1,26) = 10.83$, $p < 0.05$). Likewise, total errors were analyzed in a one-way ANOVA and there was a main effect of treatment for total errors ($F(1, 26) = 13.50$, $p < 0.05$). Throughout five sessions of reversal, the group that got MK-801 had significantly more perseverative and total errors. This reveals that MK-801 impairs the ability to shift away from the previously learned acquisition of the tactile discrimination. There was no main effect for regressive errors, revealing that MK-801 did not significantly effect the ability to learn the new reversal of the task; they were able to learn the task, however they started learning it after a longer "perseverative phase". The MK-801 group reached trials to criterion at about 160 trials on average, and the Saline group reached trials to criterion at about 130 trials on average. It is clear that the MK-801 group took longer to learn the reversal of the task, however it was not significant at a level of $p < 0.05$.

Comparison of Errors in Acquisition

Analysis of this non-spatial discrimination task versus a spatial discrimination task revealed that the non-spatial task presented in this study is significantly harder to acquire and reverse then the spatial task examined in Chadman et al. (2006). All errors in acquisition are considered to be regressive errors since there

is not previously learned response for the subject to perseverate on. Thus, all errors are accounted for in the measure of total errors. A factorial ANOVA of acquisition sessions revealed a main effect of type of task for trials to criterion, ($F(1, 38) = 148.80$, $p < 0.001$), and total errors, ($F(1, 38) = 148.80$, $p < 0.001$). Overall, the acquisition of the non-spatial task was significantly harder to learn, as it took more time and significantly more errors were made during testing. There was an additional main effect of treatment for regressive errors, ($F(1, 38) = 4.91$, $p < 0.05$). The subjects that received MK-801 in both experiments made significantly more regressive errors then subjects that received Saline.

Comparison of Errors in Reversal

A factorial ANOVA of reversal sessions reveal a main effect of type of task for trials to criterion, ($F(1, 43) = 116.42$, $p < 0.001$), perseverative errors, ($F(1, 43) = 134.47$, $p < 0.001$), regressive errors, ($F(1, 43) = 224.07$, $p < 0.001$), and total errors, ($F(1, 43) = 390.20$, $p < 0.001$). Reversal of the non-spatial task was significantly harder for subjects to learn; it took significantly more trials to learn, and they made significantly more errors then subjects in the spatial task. There was a main effect of treatment for perseverative errors, ($F(1, 43) = 7.08$, $p < 0.02$), and total errors, ($F(1, 43) = 8.34$, $p < 0.006$). There was also an interaction of treatment x type of task for perseverative errors, ($F(1, 43) = 4.81$, $p < 0.05$), and total errors, ($F(1, 43) = 7.32$, $p < 0.01$). In both cases, subjects that received MK-801 in the non-spatial task made more errors then those that received saline in the non-spatial task, while subjects that received MK-801 or Saline in the spatial task did not significantly differ. In

conclusion, task difficulty may play a large role in tasks evaluating NMDA receptor antagonism.

Chapter 8

DISCUSSION

Two experiments were performed to examine the effects of NMDA receptor antagonism on an appetitive T-maze tactile discrimination task in weanling rats. Experiment 1 found that MK-801 administered only during reversal significantly impaired performance throughout five sessions of reversal of the tactile discrimination task. This confirms the hypothesis that NMDA receptors are involved in reversal learning and extends this involvement to non-spatial tasks. Experiment 2 was designed to test the effects of MK-801 in acquisition in order to rule out performance effects as a cause of the deficit in reversal. In contrast to the hypothesis that acquisition would not be affected, Experiment 2 showed that MK-801 did impair learning of the initial discrimination of the task as well.

Studies on NMDA receptor antagonism in reversal learning in weanling rats have found various results. The effects of several doses of MK-801 were examined in a spatial discrimination task (Chadman et al., 2006). A dose of 0.06 mk/kg and 0.1 mg/kg impaired reversal learning, but not acquisition, while a dose of 0.18 mg/kg marginally impaired acquisition as well as impairing reversal learning of the task. Furthermore, reversal learning was found to be more impaired when MK-801 was received only in reversal rather than during both acquisition and reversal, thus spatial reversal learning is more sensitive to MK-801 then acquisition (Chadman et al, 2006). The present study did not find the effect that reversal is more sensitive then acquisition to MK-801.

NMDA receptor antagonists impair reversal learning in adult animals as well as weanlings. MK-801 given to adult mice during reversal in a spatial water T-maze task are impaired on reversal in a dose-dependant manner (Bardgett et al., 2003). This is similar to the findings in this study and in Chadman et al. (2006) where weanling rats were impaired on reversal of a spatial T-maze task in a dose dependant manner. An olfactory discrimination task in a Y-maze has been used to investigate the effect of MK-801 on acquisition and reversal of a non-spatial discrimination reversal task (Griesbach et al., 1998). This task is similar to the tactile discrimination reversal task in the present experiment in the fact that stimuli alternated in a quasi-random order to eliminate the relevance to reinforcement of any spatial cue. Both tasks are viewed as harder for the rats to learn, as they take many more sessions for acquisition of the task, as well as reversal of the task, than simple spatial tasks. Furthermore, Salazar et al., (2003) suggest that the particular brain areas involved in acquisition may vary according to the difficulty level of the task. A comparison of the present study and data from Chadman et al. (2006) revealed that the non-spatial discrimination task is significantly more difficult then the spatial discrimination task. The non-spatial discrimination task had higher trials to criterion, perseverative, regressive, and total errors. The difficulty of the present tactile discrimination reversal task may lead to the use of additional neuropsychological mechanisms, or additional brain regions to learn the task as suggested by Salazar at al. and Griesbach et al. In tests that examine learning and memory, task difficulty may play a large role and need to be carefully incorporated in interpreting results.

Several studies have been conducted on NMDA receptor antagonism in non-spatial reversal tasks. Highfield et al. (1996) found that MK-801 hinders patterned

single alternation, a task that has to do with working memory, in pre-weanling rats. This suggests that NMDA receptor antagonism may inhibit working memory as early as 16 days of age. Additionally, in Griesbach et al. (1998) when MK-801 was given during both acquisition and reversal, it only impaired performance during acquisition. Similarly, the present study found that MK-801 impaired acquisition of tactile discrimination, suggesting that NMDA receptors do play a role in the acquisition of non-spatial discrimination tasks. Furthermore Griesbach et al. found that when MK-801 was given only during reversal, it did impair performance, and MK-801 treated subjects learned the reversal task more slowly. This suggests that when NMDA receptors are antagonized, the affected neurons will cause the subjects to perseverate on the behavior learned in the acquisition of the odor discrimination, making it harder to learn reversal. In the present study, subjects that received MK-801 were able to learn the reversal task, however they performed significantly worse then controls. This also supports the idea that NMDA receptors are involved in suppression of previously learned repetitive behaviors and when these receptors are antagonized this suppression is impaired. In humans, NMDA receptors are involved in several clinical disorders. Stereotyped, repetitive behavior is one of the main behavioral impairments that individuals with autism possess (Carlsson, 1998). This similarity in behavior adds to the validity of animal models of autism and may be relevant to the neurological processes involved in the disorder.

Studies that evaluate spatial and non-spatial tasks in adult animals show that non-spatial tasks may use more complex, or different mechanisms then spatial tasks (Wishaw, 2004, Oliveira et al., 1997, Naghdi and Harooni, 2005, Winocur et al., 2006, and Murray et al., 1995). Murry et al. (1995) tested adult rats on a spatial and a

visual discrimination reversal task in a Y-maze. Spatial discrimination reversal was impaired while acquisition of the discrimination was left intact. This is similar to the findings of Chadman et al. (2006). However, both acquisition and reversal of the visual discrimination task were impaired by MK-801 administration, which is similar to the results found in the present study (Murry et al., 1995). In contrast to the present study, MK-801 treated rats in both tasks did not have perseverative problems during reversal but regressive problems. They were able to overcome negative transfer from acquisition of the task but had a harder time acquiring the new task (Murry et al., 1995). The present study found the same pattern of results, supporting the idea that NMDA receptor antagonism is involved in non-spatial as well as spatial tasks, and may play a role in acquisition in addition to reversal of non-spatial tasks.

A study by Wishaw (2004) shows that adult rats with lesions in the visual cortex were significantly impaired on learning a spatial task in a swimming pool. However, if subjects were given non-spatial training prior to surgery, they were able to learn the task. This suggests that rats with lesions to the visual cortex are able to remember non-spatial strategies and use them when their mechanisms of spatial learning are impaired. Thus, different brain regions may be involved in non-spatial strategies for learning. Another study showed that hippocampal lesioned adult rats are impaired in reversal of an egocentric T-maze task with no spatial cues, and they are also impaired on the reversal of a task with spatial cues (Oliverira et al., 1997). This suggests that the hippocampus plays a role in both spatial and non-spatial learning strategies. Interestingly, hippocampal lesioned subjects learned the acquisition of the task as well as controls when spatial cues are present and when they are eliminated. A transfer trial when the T-maze was turned 180 degrees examined the type of strategy

used by looking at whether subjects turned to the same place in the room, or the same place in the maze. Subjects with hippocampal lesions tended to turn to the same side of the maze, using their body position rather then spatial cues even when the spatial cues were available (Oliveira et al., 1997). The hippocampus has been found to be involved in spatial learning, and when it is lesioned, subjects are not able to use spatial cues even when they are available. This suggests that animals are able to use other brain regions and different strategies for non-spatial learning compared to spatial learning. There has also been a study done on the effects of a serotonin receptor antagonist on spatial and non-spatial learning (Naghdi and Harooni, 2005). A 5-HT 2A/2C receptor antagonist (ritanserin) and a 5HT3 antagonist (granisetron) injected into the hippocampus affected spatial but not non-spatial learning in a Morris water maze task. During spatial trials, ritanserin impaired subject's performance while granisetron enhanced subject's performance. Interestingly, performance on a non-spatial trial that is given after spatial training does not differ from controls (Naghdi and Harooni, 2005). This gives evidence to the idea that different neurological mechanisms are used in spatial and non-spatial tasks since drug treatment effected spatial trials but did not effect non-spatial trials in comparison to controls.

In contrast, another study has shown that spatial learning is impaired relative to non-spatial learning. Mice that were dosed with methotrexate and 5 fluorouracil, a form of chemotherapy treatment that causes cognitive deficits in humans, are sensitive to hippocampal damage (Winocur et al., 2006). They were tested in a Morris water maze on both spatial and non-spatial tasks. Drug treated mice were impaired on the spatial task, they made more errors had longer latencies to find the platform then controls. In two different non-spatial tasks, drug treated mice were not

impaired relative to controls. In the first task, mice were cued by a bar hanging over the location where the platform laid. In the second task, subjects had to discriminate between a black and white side of a water T-maze that were alternated on a semi-random schedule similar to the present study in order to find a platform at one end of a water t-maze. This finding that non-spatial learning is unaffected while spatial learning is impaired gives further support that there are different mechanisms involved in each.

There are several psychological processes that are apparent in this experiment. Throughout reversal, the MK-801 group was slower to learn the task, and did not reach the same level of correct choices as the control group. Both groups began at a very low percent correct level due to proactive interference from the acquisition of the task (Bouton, 1993). All subjects initially perseverated on the response they learned to be rewarding during acquisition sessions. The previously learned task interfered with learning the reversal of the task due to the negative transfer of information from the acquisition of the task, which is important to discrimination reversal learning. This suggests that there is a performance interference mechanism that plays a role in the disruption of discrimination reversal learning. This mechanism is believed to occur during performance output, rather then during learning. However, different types of memory tasks have different extents of sensitivity to changes in context (Bouton, 1993). In this experiment, proactive interference is stronger in the group treated with the NMDA antagonist. This could be due to stronger performance interference mechanisms or it could be due to the impaired learning ability that exists throughout reversal.

One theory explains that a change in context between acquisition and reversal of a task aids in learning the reversal of the task. This is because the context

shift signals that something is different and therefore proactive interference from the acquisition of the task does not inhibit reversal learning as much. Pagani et al. (2005) found that a context-shifted group was released from proactive interference earlier then a non-shifted group, and that spatial context changes were a more salient cue than a non-spatial mesh tactile cue. In the present study, a mesh tactile cue is used with no change in context, only change in stimulus the subject had to learn to follow. The NMDA receptor antagonist caused the animals to perseverate more than saline controls on the previously learned acquisition of the task, and they were released from proactive interference later in training than controls. This also has relevance to the clinical disorder autism. It was found that children with autism are impaired on the reversal of a spatial discrimination task (Coldren & Halloran, 2003). Similar to the present study, the children would persevere on the previously learned acquisition of the task and make more errors during reversal then controls. Both the subjects in this study and the children with autism showed cognitive inflexibility in learning the reversal of the task compared with controls.

It is important to examine which areas of the brain are involved in problems of learning and cognitive inflexibility. There is evidence that the hippocampus, prefrontal cortex, and striatum are involved in discrimination reversal (Bardgett et al., 2003). There is a deficit in ability to reverse a spatial water T-maze task in mice with large hippocampal lesions, it takes more trials for the mice to learn the task. This suggests that the hippocampus is important for storing and encoding memories that allow reversal learning (Bardgett et al., 2003). Furthermore, hippocampus lesions have been found to impair working memory. However, pre-training on a working memory task before creating a lesion seems to allow the brain

ample time to alter it's strategy for working memory, or train other areas of the brain to take over the processes necessary for working memory (Columbo et al., 1989). Salazar et al (2003) found that medial prefrontal lesions did not impair acquisition of a visuospatial task in an operant chamber but they did inhibit the ability of rats to learn reversal of the task. This suggests that inhibitory mechanisms that aid in reversal learning are present in the medial prefrontal cortex, and these mechanisms are impaired with lesions. Furthermore, the striatum is believed to play a role in controlling the switching of behavioral strategies on a task. An injection of an NMDA receptor antagonist, AP-5, in the dorsomedial striatum result in impaired reversal discrimination in a cross maze (Palencia & Ragozzino, 2004). This shows that the striatum may mediate some of the mechanisms involved in reversal learning. All of these brain regions may interact to result in the deficits seen in this study.

The present study adds to the literature on the role of NMDA receptors in development of weanling rats. Specifically, in a non-spatial discrimination reversal learning of weanling rats, which is relevant to neurological and cognitive disorders in learning. Intraperitoneal injections of the non competitive antagonist MK-801 are challenging to interpret because the drug diffuses into many different regions and affects them at different rates (Morris and Davis, 1994). Infusing an NMDA receptor antagonist into specific brain regions would yield more clarity in the issue of neurological activity in discrimination reversal learning. The study reveals that task difficulty has large implications in non-spatial discrimination reversal learning as well. Testing on various other non-spatial tasks would shed light on the limitations of task difficulty on learning non-spatial tasks.

Bibliography

Bardgett, M. E., Boeckman, R., Krochmal, D., Fernando, H., Ahrens, R., Csernansky, J. G. (2003). NMDA receptor blockade and hippocampal neuronal loss impair fear conditioning and position habit in C57B1/6 mice. *Brain Research Bulletin, 60,* 131-142.

Bashir, A. I., Berretta, N., Bortolotto, Z. A., Clark, K., Davies, C. H., Frenguelli, J. H., Potier, B., Collingridge, G. L. (1994). G.L. Collingridge & J. C. Watkins (Eds.), NMDA receptors and long-term potentiation in the hippocampus. *The NMDA Receptor* (pp. 294-312). New York: Oxford University Press.

Brown, K. L., Pagani, J. H., & Stanton, M. E. (2005). Spatial conditional discrimination learning in developing rats. *Developmental Psychobiology, 46,* 97-110.

Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of pavlovian learning. *Psychological Bulletin, 114,* 80-99.

Carlsson, M. L. (1998). Hypothesis: Is infantile autism a hypoglutamatergic disorder? Relevance of glutamate-serotonin interactions for pharmacotherapy. *Journal of Neural Transmission, 105,* 525-535.

Chadman, K. K., Watson, D. J., & Stanton, M. D. (2006). NMDA Receptor antagonism impairs Reversal Learning in Developing Rats. *Behavioral Neuroscience, 120, 1071-83*

Coldren, J. T., & Halloran, C. (2003). Spatial reversal as a measure of executive functioning in children with autism. *Journal of Genetic Psychology, 164,* 29-41.

Colwell, C. S., Cepeda, C., Crawford, C., Levine, M. S. (1998). Postnatal Development of Glutamate Receptor-Mediated Responses in the Neostriatum. *Developmental Neuroscience, 20,* 154-163.

Columbo, P. J., Davis, H. P., Volpe, B. T. (1989). Allocentric spatial and tactile memory impairments in rats with dorsal caudate lesions are affected by preoperative behavioral training. *Behavioral Neuroscience, 103,* 1242-1250.

Freeman, J. H., Jr., & Stanton, M. E. (1991). Fimbria-fornix transections disrupt the ontogeny of delayed alternation but not position discrimination in the rat. *Behavioral Neuroscience, 105,* 386-395.

Fellows, B. J. (1967). Chance stimulus sequences for discrimination tasks. *Psychological Bulletin, 67,* 87-92.

Green, R. J. & Stanton, M. E. (1989). Differential Ontogeny of Working Memory and Reference Memory in the Rat. *Behavioral Neuroscience, 103(1),* 98-105.

Griesbach, G. S., Hu, D., Amsel, A. (1998). Effects of MK-801 on vicarious trial-and-error and reversal of olfactory discrimination learning in weanling rats. *Behavioural Brain Research, 97,* 29-38.

Goff, D. C. & Coyle, J. T. (2001). The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *American Journal of Psychiatry, 158,* 1367-1377.

Highfield, David A., Nixon, K., Amsel, A. (1996). The NMDA antagonist MK-801 affects nonspatial learning in preweanling rats. *Behavioral Neuroscience, 110,* 300-304.

Morris, R. G. M. & Davis, M. (1994). The role of NMDA receptors in learning and memory. G.L. Collingridge & J. C. Watkins (Eds.), NMDA receptors and long-term potentiation in the hippocampus. *The NMDA Receptor* (pp. 340-375). New York: Oxford University Press.

Murray, T. K., Ridley, R. M., Snape, M. F., Cross, A. J. (1995). The effect of dizocilpine (MK-801) on spatial and visual discrimination tasks in the rat. *Behavioural Pharmacology, 6,* 540-549.

Naghdi, N., Harooni, H. E. (2005). The effect of intrahippocampal injections of ritanserin (5HT2a/2c antagonist) and granisetron (5HT3 antagonist) on learning as assessed in the spatial version of the water maze. *Behavioural Brain Research, 157,* 205-210.

Oliveira, M. G. M., Bueno, O. F. A., Pomarico, A. C., & Gugliano, E. B. (1997). Strategies used by hippocampal- and caudate-putamen-lesioned rats in a learning task. *Neurobiology of Learning and Memory, 68,* 32-41.

Pagani, J. H., Brown, K. L., & Stanton, M. E. (2005). Contextual modulation of spatial discrimination reversal in developing rats. *Developmental Psychobiology, 46,* 36-46.

Palencia, C. A., & Ragozzino, M. E. (2004). The influence of NMDA receptors in the dorsomedial striatum on response reversal learning. *Neurobiology of Learning and Memory, 82,* 81-89.

Salazar, R. F., White, W., Lacroix, L., Feldon, J., While, I. M. (2003). NMDA lesions in the medial prefrontal cortex impair the ability to inhibit responses during reversal of a simple spatial discrimination. *Behavioural Brain Research, 152,* 413-424.

Shinohe, A., Hashimoto, K., Nakamura, K., Tsujiii, M., Iwata, Y., Tsuchiya, K. J., Sekine, Y., Suda, S., Suzuki, K., Sugihara, G., Matsuzaki, H., Minabe, Y., Sugiyama, T., Kawai, M., Iyo, M., Takei, N., Mori, N. (2006). Increased serum levels of glutamate in adult patients with autism. *Progress In Neuro-Psychopharmacology & Biological Psychiatry, 30,* 1472-1477.

Watson, D. J., Sullivan, J. R., Frank, J. G., & Stanton, M. E. (2006). Serial reversal learning of position discrimination in developing rats. *Developmental Psychobiology, 48,* 79-94.

Winocur, G., Vardy, J., Binns, M. A., Kerr, L., & Tannock, I. (2006). The effects of the anti-cancer drugs, methotrexate and 5-fluorouracil on cognitive function in mice. *Pharmacology, Biochemistry, and Behavior, 85,* 66-75.

Wishaw, I. Q. (2004). Posterior neocortical (visual cortex) lesions in the rat impair matching-to-place navigation in a swimming pool: a reevaluation of cortical contributions to spatial behavior using a new assessment of spatial versus non-spatial behavior. *Behavioural Brain Research, 155,* 177-184.

Wong, E. H. F., Kemp, J. A., Priestley, T., Knight, A. R., Woodruff, G. N., Iverson, L. I. (1989). The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Neurobiology, 83,* 7104-7108.