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Medial Prefrontal Administration of MK-801 Impairs T-maze Discrimination Reversal Learning in Weanling Rats

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Abstract

Several executive functions rely on the medial prefrontal cortex (mPFC) in the rat. Aspiration and neurotoxic lesions of the mPFC impair reversal learning in adult rats [1,16,34,55]. Systemic administration of MK-801, an NMDA receptor antagonist, impairs T-maze reversal learning in weanling rats but the role of mPFC NMDA receptor antagonism in this effect is not known in either adult or young animals. This set of studies showed that mPFC NMDA receptors are specifically involved in T-maze discrimination reversal in weanling rats. In Experiment 1, 26-day-old rats (P26) demonstrated a dose-dependent impairment following bilateral mPFC administration of either 2.5 or 5.0 μ g MK-801 or saline (vehicle) during the reversal training phase only. In Experiment 2, P26 rats were trained on the same task, but 4 groups of rats received bilateral mPFC infusions during acquisition only (MK-SAL), reversal only (SAL-MK), both phases (MK-MK) or neither phase (SAL-SAL). MK-801 impaired performance only when infused during reversal. This suggests that NMDA receptor antagonism in the mPFC is selectively involved in reversal learning during development and this may account for the previously reported effects of systemic MK-801 on T-maze discrimination reversal in weanling rats.

Keywords

Development; Reversal Learning; Prelimbic Cortex; MK-801; Rat

1. Introduction

The prefrontal cortex plays a significant role in cognitive control [for recent review see 21, 41]. Cognitive control is the ability to orchestrate thought and action in accordance with internal goals [42]. As described by Miller, cognitive control is the ability to anticipate the future and coordinate and direct thought and action towards a goal; as well as maintaining goal-directed behavior to the task at hand in the selection of goal-relevant actions, while suppressing goal-irrelevant actions. The prefrontal cognitive control system is responsible for working memory, but also for dynamic responding to changes in environmental contingencies (as required by set-shifting, reversal learning, and other higher-order learning tasks) [41,42]. These diverse cognitive functions (working memory, spatial attention, monitoring, set-shifting, error-

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correction, decision-making, reward evaluation) have been attributed to distinct prefrontal regions. The functions of prefrontal cortex do not work in isolation from one another, but work together to organize behavior toward a goal [21]. Thus, the cooperative nature of all cognitive and emotional functions of prefrontal cortex is important for executive function and behavior.

The prefrontal role in complex learning tasks involving changes in task contingency, as in reversal learning, may depend on a variety of neurotransmitter systems (monoaminergic, glutamatergic, etc.). In particular, the N-methyl-D-Aspartate (NMDA) receptor subtype of glutamate receptors play a substantial role in neural physiology, synaptic plasticity, and behavioral learning and memory. These roles include, but are not limited to, the molecular/cellular basis of short- and long-term memory formation and the induction and maintenance of long-term potentiation (LTP) [13,44]. Typically, these features are attributed to the hippocampus, but similar mechanisms may be present in the prefrontal cortex, or the prefrontal cortex may receive an efferent copy of this information via extensive prefrontal-hippocampal interconnections [57].

NMDA receptors are heavily concentrated in the hippocampal formation, cortex, and striatum [70,71]. These same brain regions are essential for spatial learning and memory, contextual memory, and higher-order cognitive learning tasks in adult animals and may depend on prefrontal cortical function. Different NMDA receptor subtypes show different ontogenetic profiles in the rat [58]. Expression of the NR2B and NR2D subunits is the highest at birth and declines during postnatal development; whereas the NR1, NR2A, and NR2C subunits are barely detectable at birth and increase during postnatal development [26,43]. In terms of regional expression, the NR1 subunit is necessary for a functional NMDA receptor and is expressed throughout the brain; whereas, the family of NR2 subunits are variously expressed in the forebrain, hippocampus, and cerebellum [26,43]. In general, NMDA receptor subunit expression has reached an adult-like form by the time of weaning (P21). Studies involving systemic drug administration suggest that NMDA receptors play a functional role in learning and memory during this developmental period [11,24,25,27], but little is known concerning the behavioral functions of NMDA receptors in specific brain regions [68].

Rats as young as P7–15 can acquire and reverse a position habit using suckling as a reward [23,28]. Previous work in our lab has demonstrated that NMDA-receptor antagonism by systemically administered MK-801 impaired T-maze reversal performance in P26 rats and sensitivity to MK-801 did not change developmentally in P21–30 rats [11]. To begin to determine the underlying neural substrates of this impairment, P26 was chosen for this study as an age that is representative of reversal learning during the periweanling period [49,69] and that is well suited for studying the effects of localized drug infusions on behavior [68]. The present study examined whether antagonism of NMDA receptors specifically in mPFC mimics the effects of systemic MK-801 on T-maze reversal.

The prefrontal cortex shows a similar developmental trajectory to other cortical areas in rodents, primates, and humans [64]. In the mPFC of the rat, no discernable laminated structure can be detected at P1. By the end of the first postnatal week (P7), the basic layers (II–VIb) can be distinguished; and generated cells have migrated to their destined locations between P7–10 [32,64]. Between P10–18, the adult-like laminated pattern has emerged; and cellular differentiation is typically completed by P15 [32,64]. Little changes in the cytoarchitectonic pattern between P18–30, however there are changes in volume and neuronal maturation that occur in this period [32,64]. The mPFC increases in volume from P6–P24, with an initial reduction to adult-like volume by P30, and continued volume reduction through P90 [65].

This is the first study to demonstrate behavioral effects of administration of NMDA receptor antagonists in the mPFC in developing rats. Rats with early lesions to the prefrontal cortex

often show remarkable recovery of behavioral function when tested late in ontogeny or as adults [10,20,33,35]. Recovery depends on the age and location of the injury, as well as on behavioral measures, which suggests there is a reduction in plasticity of the mPFC in response to injury after P25 [33]. Very few studies have examined the effects of mPFC lesions on behavior during ontogeny [10,20,31]. This lab has demonstrated that P10 mPFC lesions selectively impair spatial delayed alternation (SDA) but not position discrimination when tested at P19–23, but that recovery of function occurred between P27 and P33 [20], even when the delay interval was increased from 2–45 sec [10]. Thus, the mPFC is involved in some forms of spatial learning during the weanling period but this involvement can be transferred to other brain regions following injury to mPFC on PND10. In addition to identifying a regional substrate for the effects of systemically administered MK-801 on reversal learning [11], this study reexamines the role of mPFC in memory during the weanling period with a methodology that circumvents the issue of recovery of function following neonatal lesions.

Two experiments examined the effect of NMDA receptor antagonism within the mPFC on T-maze reversal learning in weanling rats. Experiment 1 evaluated dose-response effects of MK-801 administration to determine whether antagonism of prefrontal NMDA receptors impairs reversal learning in P26 rats. Experiment 2 determined if the MK-801 impairment was specific to the reversal learning phase relative to the acquisition phase by administering MK-801 before acquisition, reversal, both, or neither. Based on prior studies with systemically administered MK-801, we predicted that the highest dose of MK-801 would lead to the greatest impairment and the lowest dose of MK-801 would modestly impair reversal learning performance (Experiment 1). We also predicted that this effect of MK-801 would be selective for the reversal learning phase, sparing acquisition performance, and eliminating the potential role of “performance” or state-dependent learning effects (Experiment 2).

2. Experiment 1

Systemic administration of MK-801 selectively impairs T-maze reversal learning in weanling rats [11]. Neurotoxic lesions of the mPFC with NMDA impairs reversal learning in adult animals [1,55]. These findings are consistent with the role of the mPFC in executive function, behavioral flexibility, and other aspects of cognitive control. Neither these lesion effects nor the role of NMDA receptors in the impairment has been examined in developing rats in T-maze discrimination reversal. Experiment 1 was designed to evaluate whether MK-801 administration into the mPFC would impair T-maze discrimination reversal learning in weanling rats, and whether the impairment was dose-dependent.

Three treatment groups received 8 blocks of T-maze discrimination reversal training (4 blocks in acquisition, and 4 in reversal). All groups received bilateral infusions in the mPFC (0.5 μ l per side). Separate groups were administered a high dose of MK-801 (5.0 μ g per side); an intermediate dose (2.5 μ g per side); or vehicle (sterile saline). If prefrontal receptors are involved in the effects of systemic MK-801 administration seen previously, then the present experiment should reveal dose-related impairment of reversal learning.

2.1 Method

2.1.1 Subjects—Forty-seven (22 female, 25 male) Long-Evans rat pups derived from 22 litters served as subjects. Litters were housed in the laboratory vivarium with ad lib access to food and water on a 12:12 hr light-dark cycle (onset at 0700 hr). Litters were culled to 8 pups (as close to 4 males and 4 females as possible) on P3 (date of birth is P0). The pups were weaned on P21 and housed with same-sex littermates until P23, with uninterrupted access to food and water, until the onset of behavioral procedures (surgery) on P23. Subjects were housed individually in cages following surgery for the duration of the experiment. Pups were allowed to recover from surgery for 1 day before the onset of deprivation.

Three pups were discarded from analysis for failure to select a maze arm within 3 minutes from the start of the trial for three or more consecutive trials during the forced run acclimation session (n=2) or the first training session (n=1). The data from 4 additional pups were removed from the experiment due to equipment failure. One pup was discarded from analysis for impaired reward consumption following drug administration. Of the remaining 39 pups, 3 pups were excluded from further analysis following histological analysis of cannula placement. These pups were excluded due to incorrect cannula placements in the medial orbital cortex rather than the prelimbic cortex. Data from the remaining 36 pups are reported below.

2.1.2 Surgery—Our surgical procedure for weanling rat cannula implantation has been described previously [see 67,68]. Commercially-obtained double cannulas (Plastics One, Roanoke, VA) were implanted bilaterally under stereotaxic guidance in the brains of weanling rats under ketamine/xylazine anesthesia (52.2–60.9 mg/kg ketamine/7.8–9.1 mg/kg xylazine in a 0.7 – 0.85 ml/kg injection volume). Buprenorphine (0.03 mg/kg in a volume of 0.05 ml/100 gm) was administered subcutaneously to alleviate pain during and following the surgical procedure. The dorsal skull surface was exposed and small holes were drilled in the skull based on stereotaxic coordinates adjusted empirically from an atlas of the developing rat brain [56]. Guide cannulas were bilaterally implanted in the mPFC (AP + 9.0 mm, ML $\pm$ 0.6 mm, DV –1.9 mm). All AP and ML coordinates were based on interaural coordinates as measured from the horizontal zero plane, such that the ear bars and incisor bar were set to zero [56]. The guide cannulas were secured to the skull with Loctite (Rocky Hill, CT) and the cannula assembly was secured to the hooks implanted in the skull with dental acrylic at the end of surgery [22, 59]. Dust caps and dummy cannulas were inserted into the guide cannula to prevent obstruction until infusions were made. Following antibiotic ophthalmic ointment application, rats were then returned to their home cages with food and water. Rats were monitored and kept warm until they recovered from anesthesia. Rats received 1 day of recovery from surgery prior to the deprivation procedure that started the T-maze protocol. This amount of recovery time has been found sufficient for weanling/juvenile rats having undergone stereotaxic surgery [18,67].

2.1.3 Drugs—The experiment involved administration of the non-competitive NMDA-receptor antagonist, dizocilpine (the compound is also referred to as MK-801). MK-801 was purchased commercially from Tocris (Ellisville, MO). It was dissolved in sterile saline.

2.1.4 Drug infusion procedure—The drug infusion procedure was performed as described by Watson et al. (2009). The rats were infused 5 minutes prior to the start of each T-maze training session. Vehicle infusions were administered to all treatment groups before the acquisition session, and MK-801 or vehicle was administered respectively to the 3 groups before the reversal session. Dust caps and dummy cannulas were removed and an injection cannula was lowered through each guide cannula extending 1 mm below the guide cannula. The injection cannula was connected to polyethylene (PE-20) tubing attached to a 10 μ L Hamilton syringe mounted on a microinfusion pump. MK-801 was dissolved in sterile saline at a concentration of either 5 or 10 μ g per μ L and delivered at a rate of 0.5 μ L per minute for 1 minute, for a total volume of 0.5 μ L per side. This volume delivered either 2.5 or 5.0 μ g of MK-801 per side, for the two concentrations of MK-801, respectively. These doses and volumes were in the range of doses commonly used in the literature for learning studies in adult rats [3,39,40,72,73] and weanling rats [67,68]. The same volume of saline was used for the control infusions. One minute after infusion, the injection cannula were removed and replaced with the dummy cannulas and dust caps.

2.1.5 Apparatus—As described by Freeman and Stanton (1991), subjects were trained in one of four Plexiglas T-mazes scaled to the size of weanling rats. Briefly, the T-mazes consisted of three equal length arms: a left and a right choice arm that were perpendicular to the start

arm. The start arm was separated from a central choice point and the choice point was separated from the choice arms by pneumatically operated doors.

Computer controlled syringe pumps that dispensed light cream reward (commercially available half-and-half) were connected to small metal cups that were located at the ends of both the left and right choice arms. When subjects broke a photoelectric beam in front of the feeding cup, the computer recorded the latency to make the response, lowered the maze doors, and when appropriate, activated the syringe pump (and delivered .07 ml light cream). Intertrial interval (ITI) boxes of clear Plexiglas were used to house subjects between trials.

2.1.6 Design—T-maze discrimination reversal training consisted of a total of 96 trials over two sessions [4 12-trial blocks per session, 19]. The experimental design was a 3 (treatment) x 2 (phase) x 4 (12-trial blocks) mixed factorial design. The rewarded goal arm, maze, and sex were counterbalanced across treatment groups. Littermates were assigned to treatment groups such that a maximum of 1 male and 1 female per litter contributed to a given group.

2.1.7 Procedure—All subjects underwent the following procedure of deprivation, maze acclimation, and training. The discrimination and reversal procedures have been described elsewhere [11,19,49]. Briefly, subjects were weaned on P21, received cannula implantation on P23, were deprived on P24, acclimated on P25, and trained on P26. During acclimation training, pups learned to consume the light cream from the reward cups at the end of each choice arm during two goal-box training sessions and then to run in the maze during a forced-run session. No infusions occurred during maze acclimation.

During the T-maze discrimination and reversal sessions, infusions occurred 5 minutes before the start of each session. Subjects were trained in squads of four animals, with two pups assigned to each maze. Subjects were given a choice of maze arm such that reward was contingent upon choosing the correct arm (either right or left, counterbalanced across subsets of subjects). Animals were removed from their ITI box and placed facing forward in the start box. The experimenter began the trial by pressing a button which caused the computer to raise the door to the choice point, after a 2-sec delay the left and right choice arms were raised. Subjects ran to an open arm to trigger the photoelectric beam at the end of the arm, causing the door to the chosen arm to lower. If the correct arm was chosen .07 ml of reward was dispensed. However, if the incorrect arm was selected, subjects remained in the goal arm for a 20-second timeout and no cream was given. Subjects were trained to consume the entire reward during the 20-sec trial. The subject was returned to its ITI box at the end of the trial. The pups were run in rotation such that the ITI for a given pup was the trial time for the other pup in the squad (~ 30 seconds). The total time to run a position habit session was thus approximately 50–55 min. After acquisition (usually between 0800 hr and 0900 hr), a 5 hour interval was imposed between the start of each session to maintain adequate motivation and to eliminate the behavioral effects of morning drug administration [11] before the reversal session began (usually between 1300 hr and 1400 hr).

Reversal training was identical to acquisition in terms of procedure, except the subjects were rewarded for entrance into the opposite goal arm (i.e. if in acquisition the rewarded arm was the left; in reversal, the rewarded arm was the right). The 48 trials in each training session were run consecutively and were divided into 4 blocks of 12 trials to analyze changes in performance within training sessions. At the end of the afternoon reversal session, subjects were returned to ad libitum access to food and water.

2.1.8 Histological Analysis—The histological analysis procedure was performed as described by Watson et al. (2009). Briefly, within 24–48 hours after completion of behavioral testing, pups were deeply anesthetized with an intraperitoneal injection of a ketamine/xylazine

cocktail following a 0.5 μ L injection of 2% pontamine skyblue dye solution through each guide cannula to show the position of the internal cannula tip. The dye solution and drug solution may diffuse different distances in the weanling rat brain. However, we have previously found that diffusion of autoradiographically-labeled MK-801 was limited to a 1 mm spherical radius from the tip of the internal cannula (in all spatial planes) when infused into the dorsal hippocampus in animals sacrificed 50 min after infusion (Burman, Stanton, & Rosen, unpublished observation). These findings confirm that MK-801 remains present throughout the duration of the session. The half-life of radio-labeled MK-801 is approximately 2 hr in rat brain, so it is likely that the drug is still actively blocking receptors throughout the session interval [29,66,70]. Animals were perfused intracardially with saline followed by formalin; brains were removed and post-fixed; and the following day, brains were placed in 30% sucrose in 10% buffered formalin. After the brains sank, coronal sections (40 μ m) were taken using a cryostat (Leica CM3050 S), mounted on slides, and then counterstained with Neutral Red (1%). Slides were examined under a microscope for cannula tip placement.

2.1.9 Data Analysis—Body weight, choice run latencies, and the total percent of correct trials per block were collected for each subject during testing sessions. These measures were subjected to analysis of variance (ANOVA), as well as post-hoc paired comparisons (Newman-Keuls). The between-groups variables used in the analysis were sex (male or female), maze (1–4), treatment (2.5 μ g MK-801, 5.0 μ g MK-801, or saline), and rewarded goal arm (left or right). The within-group variables were phase (acquisition or reversal) and blocks (4 blocks of 12 trials). No effect of sex, maze, or rewarded goal arm, was found so ANOVAs are reported with data combined across these factors.

Additional measures were also used to analyze the type of errors made during reversal training, from the total errors calculation. Total errors were categorized into either perseverative errors or regressive errors [11,15,53]. *Perseverative errors* were defined as incorrect choices 3 or more times in consecutive blocks of 4 trials. Fewer than 3 errors were classified as *regressive errors*. Perseverative errors provide a measure of the inability of the subject to shift away from the previously reinforced choice response learned in acquisition; whereas regressive errors provide a measure of the ability to learn the new discrimination after response perseveration has ended [11,15,53]. Trials to criterion (10 correct responses in 12 trials) were also calculated; if a subject did not meet this criteria, it was assigned the total number of trials (48) [11].

2.2 Results and Discussion

2.2.1 Histology – Cannula Placement—Rats were included in the analysis if the cannulas were located within or near the borders of the prelimbic region of the mPFC (3 excluded, 36 included; see included cannula placements in Figure 1). Thus, there were 12 animals in the saline group, the 2.5 μ g MK-801 dosed group, and the 5.0 μ g MK-801 group, respectively.

2.2.2 Body Weight—The average weight at deprivation for subjects was 57.4 ± 0.8 g. ANOVA performed on the deprivation weight data did not reveal differences among subjects in different treatment groups ($F < 1.39$). The average body weight at the start of Session 1 was 48.0 ± 0.80 g. ANOVA performed on the Session 1 body weight data did not reveal differences among subjects in different treatment groups ($F < 0.99$).

2.2.3 Latency—A 3 (treatment) $\times$ 2 (phase) $\times$ 4 (12-trial blocks) repeated measures ANOVA performed on the latency data revealed main effects of phase, $F(1, 33) = 50.29$, $p < .0001$; blocks, $F(3, 99) = 33.32$, $p < .0001$; and Phase $\times$ Blocks, $F(3, 99) = 31.72$, $p < .0001$; but no main effects or interactions involving treatment (all F 's < 2.36). In general, reversal latencies were faster than acquisition latencies (Acq: 3.770 ± 0.138 s; Rev: 2.813 ± 0.037 s, respectively), and latency improved across blocks, especially in Blocks 1–2 of acquisition (B1: 5.347 ± 0.408

s; B2: 3.682 ± 0.174 , respectively) relative to reversal (B1: 2.942 ± 0.093 s; B2: 2.928 ± 0.084 s, respectively). There was no evidence that the drug decreased motivation to perform the task.

2.2.4 Percent Correct Choice—The percent correct choice data are shown as a function of drug treatment (Saline, 2.5 μ g, and 5.0 μ g MK-801 treated animals), and 12-trial blocks in Figure 2. A 3 (treatment) $\times$ 2 (phase) $\times$ 4 (blocks) ANOVA was performed on the acquisition and reversal data.

Figure 2 clearly shows greatly impaired performance in the 5.0 μ g MK-801 treatment groups during the reversal phase relative to the 2.5 μ g MK-801 and vehicle treated animals. The saline-administered subjects readily acquired the reversal task. In contrast, performance of the 2.5 μ g MK-801 group was moderately impaired and the 5.0 μ g MK-801 group never performed above chance levels throughout training. There were no differences in acquisition performance (as expected because all groups received SAL in acquisition).

A 3 (treatment) $\times$ 2 (phase) $\times$ 4 (blocks) mixed factorial ANOVA yielded main effects for treatment, $F(2, 33) = 11.60, p < .0002$; phase, $F(1, 33) = 46.96, p < .0001$; blocks, $F(3, 99) = 122.75, p < .0001$; Blocks $\times$ Treatment, $F(6, 99) = 6.49, p < .0001$; Phase $\times$ Treatment, $F(2, 33) = 11.19, p < .0002$. More importantly, a Phase $\times$ Blocks $\times$ Treatment interaction was found, $F(6, 99) = 7.14, p < .0001$. In general, the 2.5 μ g MK-801 and 5.0 μ g MK-801 treatment groups had worse performance relative to the vehicle treated group, performance in acquisition was better than reversal, and performance generally improved across blocks. Newman-Keuls post-hoc analysis of the Phase $\times$ Treatment interaction revealed that the 5.0 μ g MK-treated animals ($26.38 \pm 4.159\%$) had a lower percent correct choice during reversal relative to the 2.5 μ g MK- ($51.32 \pm 4.915\%$, $p < .0008$) and saline-treated animals ($68.98 \pm 4.319\%$, $p < .0001$), which also differed ($p < .012$). More importantly, Newman-Keuls post-hoc analysis of the Phase $\times$ Blocks $\times$ Treatment interaction revealed that the 5.0 μ g MK-treated animals had a lower percent correct choice during Blocks 2–4 of reversal relative to saline-treated animals (p 's $< .0001$); and Block 3–4 of reversal relative to the 2.5 μ g MK-treated animals (p 's $< .0001$). Finally, the 2.5 μ g MK-treated group significantly differed from the SAL group in Block 2–3 ($p < .003$). Thus, a dose-response effect was found—such that the 5.0 μ g MK-treated animals showed the most impaired performance throughout reversal training, with the moderately impaired 2.5 μ g MK-treated animals surpassing the performance of the 5.0 μ g MK-treated animals at the end of reversal training.

2.2.5 Error-type Analysis—In order to characterize the types of errors made after MK-801 infusion (Figure 3), independent ANOVAs were performed on the Trials to Criteria (TTC), total errors, perseverative errors, and regressive errors. For TTC, total errors, and perseverative errors, a main effect of treatment was found, [TTC: $F(2, 33) = 8.81, p < .0009$; total: $F(2, 33) = 13.39, p < .0001$; persever: $F(2, 33) = 12.28, p < .0001$]. The effect on TTC, total and perseverative errors was due to an increased number of trials to criterion and errors made by the 5.0 μ g MK-treated animals, relative to the 2.5 μ g MK- and saline-treated animals, which differed in TTC and total errors (p 's $< .05$) and marginally differed in perseverative errors ($p < .07$). No effect of treatment was found for regressive errors, $F < 2.19$.

Taken together, the results of Experiment 1 indicate that medial prefrontal NMDA receptors are involved in T-maze reversal learning, as reflected in a dose-dependent impairment of reversal performance when MK-801 is infused into mPFC only prior to the reversal learning phase.

3. Experiment 2

The purpose of Experiment 2 was to determine whether the impairment of performance by prefrontal MK-801 administration was specific to reversal learning. Experiment 2 evaluated the role of MK-801 administration on acquisition of T-maze discrimination as well as the role of a change in drug condition across acquisition and reversal phases. Impaired acquisition would suggest a generalized effect of the drug on T-maze learning or on sensory, motor, or motivational processes necessary for performance. Impaired reversal learning would suggest the drug impairs processes specifically related to the reversal phase.

The design of this study also addressed the effects of a change in drug state across the acquisition and reversal phases of training (state-dependent learning). Drug-related cues might create an internal stimulus change between phases that may release rats from proactive interference between acquisition and reversal. Proactive interference is a behavioral mechanism in which prior learning (i.e. during acquisition) conflicts with subsequent learning (i.e. during reversal) [7]. Changes in contextual cues will disrupt memory retrieval of information encoded during acquisition, thus allowing reversal learning to proceed without interference from the acquisition memory. In developing animals, changing contextual cues across phases of T-maze discrimination improves reversal performance [45,49]. The state-dependent learning hypothesis, therefore, predicts a larger impairment of reversal when MK-801 is administered before both learning phases (i.e. no change in drug condition), than when it is administered before reversal only (i.e. a change in drug condition). State-dependent learning effects would appear as enhanced reversal performance in the groups that experienced a change in drug condition across the acquisition and reversal phases (i.e. the MK-SAL and SAL-MK groups). Previous work in our lab has found that systemic MK-801 administration does not state-dependently alter reversal performance in weanling rats [11]. Therefore, if prefrontal MK-801 administration mimics the effects of systemic MK-801 administration, we would predict no state-dependent learning effects in the present experiment.

The lowest effective dose of MK-801 from the previous experiment was used in the present study. Four treatment groups received 8 12-trial blocks of T-maze discrimination reversal training (4 blocks in acquisition, and 4 in reversal). All groups received bilateral infusions in the medial prefrontal cortex (0.25 μ l per side). Groups of rats received bilateral MK-801 (2.5 μ g per side) infusions during acquisition only (MK-SAL), reversal only (SAL-MK), both phases (MK-MK) or neither phase (SAL-SAL). If mPFC NMDA receptors are specifically involved in the reversal learning phase, then mPFC MK-801 administration will not impair acquisition, but will impair reversal regardless of drug treatment during acquisition.

3.1 Method

The methods, apparatus, etc. in Experiment 2 were the same as those detailed for Experiment 1 except where noted below.

3.1.1 Subjects—Sixty-three (32 female, 31 male) Long-Evans rat pups derived from 13 litters served as subjects. The surgical procedure began on P23 for all subjects. No more than one same-sex littermate was assigned to a given experimental group. Subjects were run in squads of 4 or 8 animals, with 2 to 4 pups assigned to each maze. There was no effect of squad size on the data.

Six pups were discarded from analysis due to loose headstages following surgery before the start of the first training session. Of the remaining 57 pups, 1 pup was excluded from further analysis following histological analysis of cannula placement. This pup was excluded because damage to the medial prefrontal cortex prohibited confirmation of correct cannula placement. Data from the remaining 56 pups are reported below.

3.1.2 Design—The experimental design was a 2 (acquisition treatment) x 2 (reversal treatment) x 2 (phase) x 4 (12-trial blocks) mixed factorial design. (Sex, maze, and direction of acquisition were additional factors, but since they failed to reveal effects in any analysis, $2 \times 2 \times 2 \times 4$ ANOVAs were performed) The acquisition treatment x reversal treatment factorial yielded four main groups, designated SAL-SAL, MK-SAL, SAL-MK and MK-MK.

3.1.3 Drug infusion procedure—The drug infusion procedure was the same as Experiment 1, except that MK-801 was dissolved in sterile saline only at a concentration of 10 µg per µL. MK-801 was delivered at a rate of 0.25 µL per minute for 1 minute, for a total volume of 0.25 µL per side. This volume delivered 2.5 µg of MK-801 per side. This volume was used to infuse the 2% pontamine skyblue dye solution during histological analysis. All other procedures were the same as detailed in Experiment 1.

3.2 Results and Discussion

3.2.1 Histology – Cannula Placement—Rats were included in the analysis if the cannulas were located within or near the borders of the prelimbic region of the medial prefrontal cortex (0 excluded, 56 included; see included cannula placements in Figure 4). Thus, group sizes were as follows: SAL-SAL group (n=16), SAL-MK group (n=13), MK-SAL group (n=13), and MK-MK group (n=14).

3.2.2 Body Weight—The average weight at deprivation for subjects was 60.7 ± 0.65 g. ANOVA performed on the deprivation weight data did not reveal differences among subjects in different treatment groups (F 's < 1.41). The average body weight at Session 1 was 51.7 ± 0.65 g. ANOVA on the weight at Session 1 did not differ between treatment groups (F 's < 2.09).

3.2.3 Latency—A 2 (acquisition treatment) x 2 (reversal treatment) x 2 (phase) x 4 (12-trial blocks) repeated measures ANOVA performed on the latency data (see Table 1) revealed main effects of acquisition treatment, $F(1, 52) = 4.59, p < .037$; reversal treatment, $F(1, 52) = 7.64, p < .008$; phase, $F(1, 52) = 17.20, p < .0001$; and blocks, $F(3, 156) = 26.88, p < .0001$; as well as interactions between Phase x Blocks, $F(3, 156) = 3.94, p < .01$; Phase x Acquisition treatment, $F(1, 52) = 5.18, p < .027$; and Blocks x Reversal treatment, $F(3, 156) = 4.08, p < .008$. As in Experiment 1, reversal latencies were faster than acquisition latencies; latency improved across the early blocks in acquisition relative to reversal; and MK-801 treated animals in acquisition had faster latencies than saline-treated animals and the same effect was found for reversal treatment. Newman-Keuls analyses of both the Phase x Acquisition treatment and Blocks x Reversal treatment interaction revealed that the SAL group had slower latencies relative to the MK treated animals in acquisition ($p < .0008$), and in the early blocks of reversal (p 's $< .02$). There was no evidence that the drug decreased motivation to solve the task as the latency effects did not match the effects on percent correct choice.

3.2.4 Percent Correct Choice—The percent correct choice data are shown as a function of drug treatment (SAL-SAL, SAL-MK, MK-SAL, and MK-MK treated animals), phase, and 12-trial blocks in Figure 5. The MK-801 treatment groups showed impaired performance during the reversal phase relative to the vehicle-treated animals. Importantly, no differences in acquisition performance following MK-801 administration were found. The reversal task was readily acquired by the saline-administered subjects, regardless of acquisition treatment. In contrast, performance of the MK-801 groups was impaired during reversal training, and did not reach the same asymptotic levels as the saline-treated animals. This impairment was the same regardless of acquisition treatment.

A 2 (acquisition treatment) x 2 (reversal treatment) x 2 (phase) x 4 (12-trial blocks) mixed factorial ANOVA yielded main effects for reversal treatment, $F(1, 52) = 9.15, p < .004$; phase, $F(1, 52) = 31.03, p < .0001$; and blocks, $F(3, 156) = 130.58, p < .0001$. More importantly, ANOVA showed significant interactions between Phase x Blocks, $F(3, 156) = 10.80, p < .0001$; and Phase x Reversal treatment, $F(1, 52) = 15.73, p < .0002$. The interaction between Phase x Blocks x Reversal treatment approached significance, $F(3, 156) = 2.37, p < .07$. In general, the groups that were administered MK-801 during the reversal phase had worse performance relative to the vehicle treated groups, performance in acquisition was better than reversal, and performance steadily improved across blocks during both acquisition and reversal for all treatment groups.

MK-801 administration did not have a state-dependent effect. When the same drug treatment is administered across phases, the state-dependency hypothesis predicts greater impairment than when drug exposure changes across phases. As noted, previously a stimulus change (such as a change drug administration) between phases is expected to improve reversal performance [49]. Previous MK-801 administration during acquisition for the MK-SAL group did not improve performance during the reversal phase relative to the SAL-SAL group. Previous saline exposure in the SAL-MK group did not enhance performance relative to the SAL-SAL or MK-MK groups. Thus, state dependency effects, such as a shift in context resulting from a change in drug cues, cannot explain our effects. Our main conclusion is that reversal learning, not initial acquisition, is more sensitive to NMDA-receptor antagonism within the medial prefrontal cortex.

3.2.5 Error-type Analysis—In order to further characterize the performance after MK-801 infusion during reversal training, independent ANOVAs were performed on the TTC, total errors, perseverative errors, and regressive errors data (Figure 6). Because there were no main or interactions effects involving acquisition treatment, data are shown pooled across this factor. For TTC, total errors, and perseverative errors, a main effect of reversal treatment was found [TTC: $F(1, 52) = 9.82, p < .003$; total: $F(1, 52) = 21.98, p < .0001$; persev: $F(1, 52) = 17.73, p < .0001$]. No other main effects or interactions with acquisition treatment were significant (F 's < 1.69). The impairment of TTC, total, and perseverative errors was due to an increased number of trials and errors made by the MK-treated animals, relative to the saline-treated animals. No main effects or interactions were found for regressive errors (F 's < 0.24). MK-801 treatment during the reversal phase enhanced the amount of perseveration on the direction trained in acquisition, which generally impaired reversal performance relative to vehicle treated animals.

4. General Discussion

Two experiments evaluated the effects of mPFC administration of an NMDA receptor antagonist on T-maze discrimination reversal learning in weanling rats. Experiment 1 demonstrated that mPFC administration of MK-801 disrupted reversal learning performance in P26 rats. A dose-dependent effect was found such that 2.5 μ g MK-801 moderately impaired reversal performance and 5.0 μ g MK-801 severely impaired reversal learning. Experiment 2 determined that the MK-801 (2.5 μ g) impairment was specific to the reversal learning phase. Importantly, learning was completely intact during the acquisition phase following NMDA receptor antagonism. Thus, disruption of sensory, motor, motivational, or general learning processes cannot explain the reversal learning deficit. State-dependent learning effects, also cannot explain the reversal learning impairment. MK-801 treatment had no effect on body weights (Experiment 1 and 2) and caused slightly faster choice run latencies in both acquisition and reversal (Experiment 2). The significance of these numerically small differences in latency for choice accuracy is unclear because effects were seen during both training phases whereas

impaired accuracy was seen only during reversal. In any case, there was no evidence that the drug decreased motivation to solve the task.

Discrimination and reversal has been demonstrated in P7–30 rat pups using Y-mazes [28], T-mazes [23,45,49], and olfactory cues [25]. The reversal learning phase, but not acquisition, is NMDA receptor dependent in weanling rat pups using systemic MK-801 administration [11, 25]. Amsel and colleagues reported that a moderate systemic dose of MK-801 (0.05 mg/kg, ip) disrupted olfactory discrimination and its reversal in P22–28 rats [25]. T-maze discrimination reversal learning was selectively impaired by administration of a high dose of MK-801 (0.10 mg/kg) before the reversal learning phases in P21–30 rat pups. Administration of the same dose of MK-801 prior to the acquisition phase did not impair initial learning of the position habit [11]. The reversal impairment was not due to performance related deficits in sensory, motor, or motivational processes, but was specific to the reversal learning phase, leaving the initial acquisition intact. The current findings suggest that mPFC effects of MK-801 may account for the effects of systemic drug administration established previously [11].

The evidence that the initial acquisition of the position habit was intact in Experiment 2 suggests that specific NMDA receptor targeting of the mPFC by MK-801 underlies the reversal deficit. Non-NMDA mechanisms (possibly dopamine or acetylcholine) and/or other brain regions (nucleus accumbens or basal forebrain nucleus) may account for acquisition performance; but reversal learning is NMDA receptor dependent, at least in the mPFC in P26 rats. The fact that reversal learning is impaired, but acquisition is intact is likely due to the fact that there are different task demands in each phase. Reversal learning of a discrimination is cognitively more demanding than initial acquisition of a discrimination [15]. Reversal learning is dependent upon three behavioral processes: 1) memory of the initially acquired response, 2) learned suppression of this initially acquired response, 3) learning the new (competing) response. Thus, prefrontal NMDA receptors are not needed for the initial learning phase in acquisition, but when task demands are more complex in reversal, this brain region becomes necessary for successful performance of the task in weanling rats. It is generally well-accepted that the theoretical function of the prefrontal cortex is cognitive control [for recent review see 21,41]. Cognitive control is the ability to orchestrate thought and action in accordance with internal goals [42]. Cognitive control is a broad term that encompasses a variety of executive functions of the prefrontal cortex, which include: 1) attention (subcategories of set, working memory, and interference control); 2) planning; 3) decision-making [21]. The executive functions of the prefrontal cortex are applicable across human, primate, and rodent studies [8,36,62]. Medial prefrontal lesions in adult rats have been shown to disrupt spatial learning and tasks that involve a change in task contingency—such as attentional set-shifting and reversal learning [reviewed in 62]. NMDA receptors in the mPFC are likely to be specifically involved in reversal learning, but not acquisition because reversal learning is cognitively more demanding and dependent on executive function than is initial acquisition.

These findings extend our previous developmental work by showing, for the first time, that in addition to the hippocampus [68], mPFC function is also specifically involved in reversal learning in weanling rats. The current finding that the mPFC is necessary for T-maze reversal learning during development informs animal models of autism, fetal alcohol syndrome, and schizophrenia, which typically show deficits in spatial working memory, behavioral flexibility, and executive function [12,30,60]. To better inform these animal models, it is critical to understand how NMDA receptor contribution in a variety of brain regions affects these behavioral learning paradigms in developing animals, as well as how these structures interact and emerge across ontogeny. NMDA receptor hypofunction is a potential mechanism that may underlie the deficits seen in schizophrenia and autism [9,38]. Understanding how different brain structures and neurochemical systems are involved in reversal learning across development will contribute importantly to animal models of neurodevelopmental disorders.

This is the first study to directly evaluate the effects of mPFC infusion of NMDA receptor antagonists on learning and memory in developing rats. We have demonstrated that mPFC NMDA receptors are necessary for reversal learning in P26 rats. Our data is consistent with the role of the mPFC that has been confirmed previously with lesion data in adult rats. Early reports using prefrontal aspiration lesion techniques demonstrate a severe impairment in spatial reversal retention [16,48] as well as spatial serial reversal using a Grice box [34]. In an interesting developmental analysis of spatial reversal performance, Kolb and colleagues found that mPFC lesions on P5 or P9 did not impair spatial reversals when tested as adults. P25 lesions moderately impaired performance, and severe deficits were seen after mPFC lesions on P35, 40, and 100 [33]. The present study in P26 rats is consistent with these findings. Future studies need to investigate the role the mPFC plays in reversal learning at different developmental ages. Advances in prefrontal lesion techniques, especially the use of excitotoxic agents that spare fibers of passage, have also demonstrated impaired reversal learning of 2-choice lever pressing in adult rats [1,14,55]. The current reversal findings have been less consistent with studies involving temporary pharmacological inactivation [17,50,51,54] and excitotoxic lesioning [6] of the mPFC. These studies primarily showed that the prelimbic subregion of mPFC was not necessary for response reversal learning in a lever-pressing response discrimination [6,17] or in a modified plus-maze [50,51,54] in adult rats. Although there were other parametric differences between the studies (testing apparatus, methodological procedures, and pharmacological manipulations), it is likely that differences in the age of the subjects tested accounts for the discrepancies across these studies. Thus, it may be possible that in weanling animals the medial area of prefrontal cortex is necessary for reversal learning, but it is no longer necessary in adulthood. A similar concept that forebrain regions may be necessary for “simpler” discriminations in young rats and switch to more “complex” functions in adult rats has been proposed previously [37]. Ethanol exposure (that targets the hippocampus) impaired performance of an odor-digging discrimination task in adolescent rats but was ineffective in adults. Additional empirical work is required to fully characterize age differences in the contribution of prefrontal NMDA receptors to this reversal deficit.

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Figure 1. Schematic representation of cannula placements targeted to the prelimbic area of the mPFC in Experiment 1. Coordinates represent distances anterior to Bregma (Paxinos & Watson, 2005).

Figure 2.

Mean (+SE) percentage of correct responses for the three treatment groups beginning on P26 in Experiment 1 as a function of training phase (acquisition or reversal), 12-trial blocks, and dose. During the reversal phase only, the mPFC treatment groups were vehicle (SALINE: open circles), or one of two drug doses (2.5 µg MK-801: closed circles; 5.0 µg MK-801: closed squares). All treatment groups received saline infusions during acquisition. Dashed line at 50 percent indicates chance performance.

Figure 3. Analysis of trials to criterion (TTC) and error types during reversal for P26 rats as a function of dose of MK-801 in Experiment 1. Mean (+SE) for TTC (A), total errors (B), perseverative errors (C), and regressive errors (D).

Figure 4. Schematic representation of cannula placements targeted to the prelimbic area of the mPFC in Experiment 2. Coordinates represent distances anterior to Bregma (Paxinos & Watson, 2005).

Figure 5.

Mean (+SE) percentage of correct responses for the four MK-801 (2.5 μ g) treatment groups in Experiment 2 beginning on P26 as a function of training phase (acquisition or reversal), 12-trial blocks, and treatment. The mPFC treatment groups were saline-saline (SAL-SAL: open circles), saline-MK-801 (SAL-MK: closed circles), MK-801-SAL (MK-SAL: open triangles), or MK-801-MK-801 (MK-MK: closed triangles). Dashed line at 50 percent indicates chance performance.

Figure 6.

Analysis of trials to criterion and error types during reversal for P26 rats as a function of dose of MK-801 in Experiment 2. Differences were not found between a first (SAL-MK) or second (MK-MK) infusion of MK-801 during reversal, thus the SAL-MK and MK-MK treatment groups were pooled into Group MK (open bars), and the saline groups (MK-SAL and SAL-SAL) were pooled into Group SAL (closed bars). Mean (+SE) for TTC (A), total errors (B), perseverative errors (C), and regressive errors (D).

Table 1

Mean (+SE) of choice run latencies for the four treatment groups beginning on P26 in Experiment 2 as a function of training phase (acquisition or reversal), 12-trial blocks, and dose.

	ACQUISITION				REVERSAL				<i>n</i>
	Block 1	Block 2	Block 3	Block 4	Block 1	Block 2	Block 3	Block 4	
SAL-SAL	4.133 ± 0.444	3.382 ± 0.238	2.894 ± 0.083	2.773 ± 0.071	3.173 ± 0.184	2.869 ± 0.109	2.734 ± 0.072	2.641 ± 0.034	16
SAL-MK	3.340 ± 0.194	3.161 ± 0.306	2.718 ± 0.061	2.745 ± 0.129	2.738 ± 0.039	2.660 ± 0.067	2.560 ± 0.032	2.528 ± 0.028	13
MK-SAL	3.185 ± 0.066	3.017 ± 0.243	2.767 ± 0.096	2.587 ± 0.034	3.132 ± 0.124	2.925 ± 0.159	2.638 ± 0.041	2.573 ± 0.022	13
MK-MK	3.034 ± 0.164	2.771 ± 0.120	2.679 ± 0.055	2.666 ± 0.054	2.677 ± 0.063	2.644 ± 0.036	2.579 ± 0.042	2.595 ± 0.027	14