Published in final edited form as:

Behav Neurosci. 2006 October ; 120(5): 1071–1083. doi:10.1037/0735-7044.120.5.1071.

NMDA Receptor Antagonism Impairs Reversal Learning in Developing Rats

Kathryn K. Chadman, Deborah J. Watson, and Mark E. Stanton

Department of Psychology, University of Delaware.

Abstract

Four experiments examined the effect of dizocilpine maleate (MK-801), a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, on reversal learning during development. On postnatal days (PND) 21, 26, or 30, rats were trained on spatial discrimination and reversal in a T-maze. When MK-801 was administered (intraperitoneally) before both acquisition and reversal, 0.18 mg/kg generally impaired performance, whereas doses of 0.06 mg/kg and 0.10 mg/kg, but not 0.03 mg/kg, selectively impaired reversal learning (Experiments 1 and 3). The selective effect on reversal was not a result of sensitization to the second dose of MK-801 (Experiment 2) and was observed when the drug was administered only during reversal in an experiment addressing state-dependent learning (Experiment 4). Spatial reversal learning is more sensitive to NMDA-receptor antagonism than is acquisition. No age differences in sensitivity to MK-801 were found between PND 21 and 30.

Keywords

spatial discrimination; reversal; MK-801; development

The NMDA subtype of glutamate receptor plays an important role in synaptic plasticity and learning and memory. NMDA receptors are found throughout the brain, with the highest concentrations of [³H]-MK-801 binding in the frontal cortex, striatum, and the hippocampus (Porter & Greenamyre, 1995), brain areas associated with learning and memory. There is compelling evidence that NMDA receptors are necessary for the induction of long-term potentiation, a long-lasting increase in synaptic efficacy (Bliss & Lomo, 1973; Douglas & Goddard, 1975; Lynch & Baudry, 1984) that is thought to underlie some forms of learning. NMDA antagonists block induction of long-term potentiation in the hippocampus (Collingridge, Kehl, & McLennan, 1983); therefore, blockade of NMDA receptors has profound effects on synaptic plasticity and learning and memory. NMDA receptors have been shown to be essential for spatial learning and memory (Nakazawa, McHugh, Wilson, & Tonegawa, 2004).

The glutamate receptor system is thought to be a major signaling pathway for neuronal migration during brain development. Neonatal rats administered NMDA antagonists during

the first 2 weeks of life develop abnormal axonal arborization (Simon, Prusky, O'Leary, & Constantine-Paton, 1992). NMDA receptor binding measured across ontogeny in the rat neostriatum showed increases in dizocilpine maleate (MK-801) binding across postnatal days (PND) 3 to 21, with a peak at PND 28 and a decline to adult levels by PND 60 (Colwell, Cepeda, Crawford, & Levine, 1998). In the rat, at birth NMDA receptors are made up of NR1 and NR2B subunits, with the gradual inclusion of NR2A over the first 5 weeks of life (Bear & Rittenhouse, 1999; Quinlan, Olstein, & Bear, 1999). Pronounced long-term depression has been induced in hippocampal slices from rats during the 1st week of life and shows a decline during development, whereas reliable long-term potentiation continues to develop at least through the 3rd week of life (Dudek & Bear, 1993). Long-term potentiation in the hippocampus and visual neocortex is enhanced during early ontogeny in the rat, peaking around PND 15 before stabilizing at lower levels during later stages of development and adulthood (Teyler, 1989). In contrast to this large literature on NMDA involvement in neural plasticity during brain development, much less is known about NMDA involvement in learning and memory during ontogeny (Griesbach, Hu, & Amsel, 1998; Highfield, Nixon, & Amsel, 1996; Lincoln, Coopersmith, Harris, Cotman, & Leon, 1988).

To further address this question, the present study examined the effects of the NMDA antagonist MK-801 on reversal learning during the weanling period in rats. Reversal of discrimination learning is thought to involve more processes than acquisition of discrimination (McAlonan & Brown, 2003; White, 2004) and may be a measure of cognitive flexibility (Coldren & Halloran, 2003; McAlonan & Brown, 2003; White, 2004). Reversal of a spatial discrimination requires the inhibition of the learned response to a previously learned set of contingencies, acquisition of a response to the new set of contingencies, and memory processes such as proactive interference and processing of contextual cues (Watson, Sullivan, Frank, & Stanton, 2006). It has been shown that blockade of NMDA receptors impairs spatial reversal learning without affecting acquisition in adult rats (Murray, Ridley, Snape, & Cross, 1995; Palencia & Ragozzino, 2004). Nothing is known about how NMDA antagonism affects spatial reversal during development. There is evidence that MK-801 blocks the reversal of odor discrimination in weanling rats (Griesbach et al., 1998) and inhibits learning of a patterned single alternation task (Highfield et al., 1996). Therefore, it is known that NMDA receptor antagonism does alter learning in preweanling and weanling rats; however, its effect on spatial reversal learning has not been examined. The current study contributes to the developmental literature on learning by examining the effects of MK-801 on spatial reversal learning at specific points in development and by studying many aspects of MK-801 on behavior, such as tolerance, sensitization, and state-dependent learning.

Four experiments examined the effects of NMDA receptor antagonism on reversal learning of a spatial discrimination in weanling rats. The first experiment examined several doses of MK-801 to determine whether there is a dose that impairs reversal but not acquisition in PND 26 rats. The second set of experiments examined sensitization to MK-801 to determine whether the effects on reversal learning are specific to the task or due to sensitization to two successive doses of MK-801. The third set of experiments expanded the findings from Experiment 1 to two other weanling ages, PND 21 and 30. Lastly, the effect of MK-801 at

different stages of training was tested in three ages of weanling rats, PND 21, 26, and 30, by administering MK-801 before acquisition, reversal, both, or neither (vehicle).

Experiment 1

The set of procedures in Experiment 1 was designed to determine the dose range of MK-801 that impairs reversal learning but has no effect on the acquisition of discrimination and therefore no effects that would disrupt general performance in this task. The doses chosen were saline vehicle and 0.056 mg/kg, 0.10 mg/kg, and 0.18 mg/kg MK-801. These doses were chosen on the basis of previous studies of weanling and adult rats. The two lower doses are in a range that is commonly used in the literature (Griesbach et al., 1998; Highfield et al., 1996; Murray et al., 1995; van der Meulen, Bilbija, Joosten, de Bruin, & Feenstra, 2003), whereas the higher dose represented a less common “upper limit” that was accompanied by modest motor effects in some rats. MK-801 or saline was administered before both acquisition and reversal. We found that 0.18 mg/kg MK-801 impaired both acquisition and reversal, 0.10 mg/kg only impaired reversal learning, and 0.056 mg/kg had no effect relative to saline vehicle controls.

Method

Subjects—Subjects were 50 Long Evans rats (25 male, 25 female) that were the offspring of 14 time-bred females. Dams were obtained from Harlan breeders (Fredrick, Maryland) between Gestational Days 5 and 12 and left undisturbed until giving birth. The age of birth was determined by checking for births during the light cycle; if litters were found, they were designated PND 0. Litters were culled to 4 males and 4 females (or as close as possible) on PND 3. The pups were left undisturbed until PND 21, except for routine cage changes. Individual dams were housed with their litters in clear polypropylene cages that measured 8” high × 18” long × 9” wide (20.3 cm × 45.7 cm × 22.9 cm) in a housing facility that was maintained on a 12-hr light–dark cycle with lights on at 7 a.m.

Pups trained on PND 26 were weaned from their mothers at PND 21 and were housed with same-sex littermates with a continual supply of food and water until experimentation. Fifty pups were deprived at PND 24 (see procedure below). The average weight at deprivation of this group was 65.4 ± 0.85 g (range = 47–75 g).

Apparatus—Four identical T-mazes, scaled to weanling/juvenile rats, were used to train subjects. The design of these T-mazes enabled a single experimenter to operate two mazes simultaneously, as described in detail elsewhere (Freeman & Stanton, 1991). Briefly, the T-mazes were constructed of clear Plexiglas covered on the outer wall with brown paper, except on the lids of the arms, and consisted of two goal boxes and a start arm, all of equal size. The start arm was separated from the goal boxes by computer-controlled, pneumatically operated doors. These doors created a choice point that separated the start arm from the goal boxes when the goal box doors were down. Illumination was provided by a single 25-W amber bulb located directly above the choice point. A small metal cup was located at the end of both goal boxes. Light cream (commercially available half-and-half) was pumped into these cups when a correct choice was made. Responses were recorded when subjects broke a photoelectric beam located near the end of the goal box. The latency

to break this beam was recorded by the computer, which also controlled the pneumatic doors and the pumps that delivered the light-cream reward. The mazes themselves were situated on a single tabletop approximately 1.5 feet (0.46 m) apart and were operated simultaneously by a single experimenter. During the intertrial interval (ITI), subjects were kept in $11.5 \times 11.5 \times 18.5$ cm “ITI boxes” made of clear Plexiglas and fitted with hinged, ventilated lids.

Drugs—MK-801 was purchased commercially from Tocris (Ellisville, Missouri) and dissolved in sterile saline prior to the experiment for administration. The volume of injection for each subject was 1.0 ml/kg, administered as an intraperitoneal injection.

Design—The design involved the between-subjects variables of treatment (0.056, 0.10, or 0.18 mg/kg MK-801) or saline vehicle. Rats were trained to choose one arm of the T-maze (left or right) during the acquisition session in the morning and the opposite arm during the subsequent afternoon reversal session. Each acquisition and reversal session consisted of four blocks of 12 trials for a total of 48 trials. These sessions occurred in the morning and afternoon, lasted for about 2 hr, and started about 6 hr apart.

In these groups, subjects were rewarded for entering the correct choice arm during acquisition and for entering the opposite goal arm in the next session. The rewarded goal arm, maze, sex, dose, and dam were counter-balanced across subjects within each treatment and behavioral group. For acquisition and reversal training, the design was a 4 (treatment) $\times$ 2 (phase) $\times$ 4 (blocks) mixed factorial design.

Procedure—The procedure was based on the general methods of Freeman and Stanton (1991). For all pups, the procedure involved deprivation, maze acclimation, training, and reversal. These phases are described as follows.

Deprivation and maze acclimation: Subjects were deprived of food, water, and maternal/sibling social contact between about 1600 and 1700 on PND 24, approximately 16 hr prior to acclimation training in the T-maze (see below). Deprivation and maze acclimation were the same as reported in Freeman and Stanton (1991) except where noted. Subjects were weighed to the nearest gram, tail marked for identification, and individually housed in opaque Plexiglas containers. Before being placed into these cages, pups were given approximately 0.07 ml of light cream by mouth. One ml of cream was also placed in a metal spoon secured to the inside of the Plexiglas cage. This pre-exposure to light cream facilitates consumption of reward during subsequent maze acclimation. Throughout the experiment, pups were fed supplementary light cream when needed in order to maintain their body weight at about 85% of their weight at the start of deprivation (see below).

Subjects were trained in squads of 6 to 8: Three to four rats were assigned to each of the two mazes. Before being trained on the position habit, all rats were exposed to the maze and taught to consume reward. Acclimation consisted of two goal-box training sessions at about 0800 and 1200 and a forced-run training session at about 1600, all on PND 25. During the goal box sessions, the subject was placed in one of the maze arms until it consumed approximately 0.07 ml reward or until 3 min had elapsed. Subjects that failed to consume reward within 3 min of being placed in the goal box were given approximately 0.07 ml by

mouth on removal from the box to facilitate goal-box training. This occurred no more than three times in any goal box session. All subjects were given six trials in each arm of the maze (e.g., left arm during the first goal box session and right arm during the second, or vice versa). The forced-run session consisted of 12 trials (6 to the left, 6 to the right in an irregular sequence) in which the subject was presented with a single goal arm and was rewarded with 0.07 ml light cream on breaking the photocell at the end of the goal arm.

Training and reversal series: Fifty rats were assigned to one of four groups at PND 26: saline vehicle ($n = 15$), 0.056 mg/kg MK-801 ($n = 11$), 0.10 mg/kg MK-801 ($n = 12$), and 0.18 mg/kg MK-801 ($n = 12$). Prior to the start of each training session, the pups were weighed and injected intraperitoneally with either saline or MK-801 in a 1.0 ml/kg volume. Following the injection, the subjects were placed in ITI boxes for 30 min prior to the start of the session, which is also where they remained between trials. During the acquisition phase of the experiment, subjects were presented with a choice of both maze arms, with reward contingent upon choosing the correct arm (either right or left, counterbalanced across subsets of subjects). The 4 pups in a squad were run in rotation such that the ITI for a given pup was determined by the time required to run the other 2–3 pups in the squad (typically 60–90 s). After completing acquisition (in approximately 2.0 hr), pups obtained supplementary cream reward to maintain 85% of body weight at deprivation and to account for unsuccessful trials in the session.

The first reversal session occurred 6 hr after the start of acquisition. Reversal training was identical to acquisition except that the subjects were now 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). At the end of the afternoon session, subjects were returned to ad libitum access to food and water.

Analysis—Data were collected from each pup for each session. These data included the weight, latency to choose, percentage correct, and number of errors for each block or session. The data were then subjected to analysis of variance (ANOVA) for both acquisition and reversal sessions. The between-groups variables used in the analysis were sex (male or female) and treatment (dose of MK-801 or saline). The within-group variables were phase (acquisition or reversal) and blocks (four blocks of 12 trials).

Results

The percentage correct data for each of four blocks of 12 trials during acquisition and reversal phases of training during this experiment are shown in Figure 1. Initial analyses indicated that there were no significant main effects of sex or of nested variables such as maze (1, 2, 3, or 4) or acquisition direction (left or right). Thus, data were pooled across these variables, and a 4 (treatment) $\times$ 2 (phase) $\times$ 4 (blocks) mixed design ANOVA was performed for acquisition and reversal.

The subjects administered MK-801 performed similarly to saline controls during acquisition but were impaired in a dose-dependent manner during reversal learning. There were main effects of treatment, $F(3, 46) = 9.77, p < .001$; phase, $F(1, 46) = 70.00, p < .001$; and block, $F(3, 138) = 174.16, p < .001$. There were also significant interactions of Phase $\times$ Block, $F(3,$

138) = 22.07, $p < .001$; Treatment $\times$ Phase, $F(3, 46) = 3.33$, $p < .05$; and Treatment $\times$ Block, $F(9, 138) = 3.69$, $p < .001$; as well as a marginally significant interaction of Treatment $\times$ Phase $\times$ Block, $F(9, 138) = 1.719$, $p < .091$. The Phase $\times$ Block interaction occurred because the subjects made fewer correct choices at the outset of reversal than at the outset of acquisition but performed similarly near the end of each session. The interaction of treatment and phase reflects the larger effect MK-801 had on reversal learning in comparison with acquisition. Newman–Keuls tests showed no significant differences between any of the groups during acquisition ($p > .05$): saline (77.58% $\pm$ 2.18), 0.06 mg/kg (71.22% $\pm$ 1.87), 0.10 mg/kg (76.04% $\pm$ 2.98), and 0.18 mg/kg (68.60% $\pm$ 3.04). Newman–Keuls tests showed significant differences between the saline (66.42% $\pm$ 3.72) and 0.18 mg/kg (37.29% $\pm$ 5.52) groups during reversal ($p < .05$). The middle two MK-801 dose groups did not differ significantly from each other or the saline or 0.18 mg/kg groups ($p > .05$): 0.06 mg/kg (54.41% $\pm$ 4.95) and 0.10 mg/kg (50.33% $\pm$ 3.43). The Treatment $\times$ Block interaction was driven largely by the effect of treatment during the reversal phase and reflected the fact that treatment effects were smaller in Blocks 1 and 4 and larger in Blocks 2 and 3.

Planned comparisons were performed on the block means for each treatment group and phase. There were significant differences among the treatment groups during the third block of acquisition and during the last three blocks of reversal. In the third block of acquisition, the 0.06 mg/kg MK-801 group made significantly fewer correct choices (73.46% $\pm$ 5.00) in comparison with the saline group (86.67% $\pm$ 3.05) and the 0.10 mg/kg MK-801 group (86.75% $\pm$ 2.99). This is the only block during acquisition where there was a difference among the treatment groups; the significance of this difference is unclear (nor was it replicated in subsequent experiments; see below). During reversal, there were no differences among the treatment groups in Block 1, but significant differences emerged in the last three blocks. MK-801 impaired learning during the last three blocks of reversal in a generally dose-dependent fashion. The saline control group consistently performed better than the MK-801 groups, and the group given the highest dose of MK-801 (0.18 mg/kg) performed the most poorly during these reversal blocks. During the second block of reversal, each of the treatment groups was significantly different from the others ($p < .05$), except the groups given the two highest doses of MK-801 (0.10 and 0.18 mg/kg), which did not differ. In the third block, the saline group made significantly fewer errors (81.67% $\pm$ 5.63) than all of the other groups ($p < .05$), and the 0.18 mg/kg MK-801 group made significantly more errors (38.92% $\pm$ 7.33) than all of the other groups ($p < .01$). The 0.06 (62.97% $\pm$ 8.30) and 0.10 mg/kg (66.67% $\pm$ 5.61) MK-801 groups were not different from each other. During the last block of reversal, again the saline group performed significantly better than the 0.06 and 0.18 mg/kg MK-801 groups ($p < .05$) but not the 0.10 mg/kg group. The 0.18 mg/kg MK-801 group was significantly different from both the saline and 0.10 mg/kg MK-801 groups ($p < .01$) but not the 0.06 mg/kg MK-801 group.

In summary, MK-801 did not impair acquisition learning compared with saline controls but did impair reversal learning in a dose-dependent manner.

Experiment 2

Experiment 2 was designed to determine whether the larger drug effects on reversal reflected sensitization to the second dose of MK-801 or a specific impairment of the reversal learning task. The largest dose of MK-801 (0.18 mg/kg) tested in Experiment 1 was chosen for this experiment. We reasoned that sensitization effects would be most likely to appear at this high dose. Therefore, if they failed to appear at this dose, they would be unlikely to be a factor at the other, lower doses of MK-801.

Method

There were four groups tested. In the first two, MK-801 or vehicle was administered prior to both acquisition and reversal; in the third and fourth groups, MK-801 or vehicle was administered prior to a morning “sham session” and again prior to an afternoon acquisition session. In this way, acquisition following 1 or 2 injections of MK-801 could be compared, and acquisition and reversal under the same conditions (afternoon, two doses of MK-801) could be compared. Administration of one or two injections of MK-801 prior to acquisition demonstrated equivalent impairments relative to vehicle. Reversal was again more impaired than acquisition under conditions that could not be attributed to drug sensitization.

Subjects—Subjects were 71 Long Evans rats (35 male, 36 female) that were the offspring of 10 time-bred females. All details concerning procurement, housing, husbandry, etc. were as described previously in Experiment 1. The average weight at deprivation of this group was 64.8 ± 0.76 g (range = 36–66 g).

Apparatus—The apparatus was the same as described in Experiment 1.

Design—The design involved the between-subjects variables of treatment (MK-801 or vehicle) and training group. One group received acquisition in the morning and reversal in the afternoon. We designated this group acq + rev. The other group received a “sham session” in the morning, followed by an acquisition session in the afternoon. We designated this condition sham + acq. All subjects received either vehicle or 0.18 mg/kg MK-801 prior to both morning and afternoon sessions.

The design was a 2 (treatment) $\times$ 2 (training group) $\times$ 4 (blocks) mixed factorial design.

Procedure—For all pups, the procedure was deprivation, maze acclimation, training, and reversal. These phases were identical to those described in Experiment 1 except as noted below.

Seventy-one rats were assigned to one of four groups at PND 26: saline acq + rev ($n = 16$), MK-801 acq + rev ($n = 19$), saline sham + acq ($n = 19$), and MK-801 sham + acq ($n = 17$). Prior to the start of each training session, the pups were weighed and placed in ITI boxes, where they remained between trials. During the acquisition phase of the experiment, subjects were presented with a choice of maze arm, and reward was contingent upon choosing the correct arm (either right or left, counterbalanced across subsets of subjects). During the sham session, in the morning for the sham + acq groups, the pups were weighed

and placed in the ITI boxes for 60–90 min under the same conditions as a real session except that they were never placed in the maze. The remaining details for the morning and afternoon sessions were as described in Experiment 1.

Analysis—Dependent measures were as described in Experiment 1. The variables examined in the analysis were sex (male or female), treatment (MK-801 or vehicle), and training group (sham + acq or acq + rev).

Results

The percentage correct data for each of four blocks of 12 trials during acquisition and reversal phases of training during this experiment are shown as a function of drug treatment and group in Figure 2. All groups acquired the discrimination as reflected by an increase in percentage correct choices across blocks, though the MK-801 treated groups made fewer percentage correct choices overall (Figure 2: filled symbols on left; open symbols in middle). During the first block of reversal, the acq + rev group made fewer correct choices than did the sham + acq group at the outset of acquisition. Both groups improved across blocks. After two administrations of MK-801, MK-801 failed to affect acquisition (Figure 2: middle, open symbols) but severely impaired reversal (Figure 2: right, closed symbols).

Separate ANOVAs involving this design were run on acquisition (after one or two doses of MK-801) and reversal (acquisition vs. reversal in separate groups receiving two doses of MK-801). For each ANOVA, initial analyses indicated that there were no significant main effects of sex or nested variables such as maze (1, 2, 3, or 4) or acquisition direction (left or right). Thus, data were pooled across these variables in the main ANOVAs. The analysis of acquisition was a 2 (drug: saline vs. MK-801) $\times$ 2 (group: sham + acq vs. acq + rev) ANOVA, which compared the four blocks of acquisition between the groups that received saline or drug once (acq + rev; Figure 2, left) or twice (sham + acq; Figure 2, middle, open symbols). There were no main effects or interactions involving the variable of number of doses before acquisition ($p > .05$), indicating that the subjects administered a second dose of MK-801 performed similarly to those administered a single dose of MK-801 before acquisition. The dose of MK-801 used for this experiment, 0.18 mg/kg, significantly impaired acquisition, as reflected in a main effect of drug, $F(1, 31) = 7.04$, $p < .05$. There was also a significant main effect of block, $F(3, 93) = 57.72$, $p < .001$, that did not interact with drug treatment or the number of injections before acquisition (both $ps > .05$). The fact that MK-801 impairs acquisition similarly whether administered once or twice before the session makes it unlikely that sensitization to the drug with repeated administration accounted for the greater sensitivity of reversal to MK-801 in Experiment 1.

A second analysis was run comparing the acquisition session from the sham + acq groups (Figure 2, middle) with the reversal session from the acq + rev groups (Figure 2, right). In this way, both groups were matched for the number of drug administrations and the time of day when the session was run. However, one group was undergoing acquisition (sham + acq), and the other was undergoing reversal (acq + rev). Again, the ANOVA was a 2 (groups) $\times$ 2 (drug) $\times$ 4 (blocks) mixed factorial. There were significant main effects of group, $F(1, 31) = 26.42$, $p < .001$; drug, $F(1, 31) = 13.35$, $p < .01$; and block, $F(3, 93) =$

58.91, $p < .001$; as well as interactions of Group $\times$ Drug, $F(1, 31) = 7.75$, $p < .01$; Group $\times$ Block; $F(3, 93) = 3.80$, $p < .05$; and Group $\times$ Drug $\times$ Block, $F(3, 93) = 4.69$, $p < .01$. Newman–Keuls analysis of the three-way interaction showed a significant difference between the sham + acq groups and the acq + rev groups ($p < .01$) during the first block of the session regardless of drug. In the second block, the saline sham + acq group was significantly different from the saline acq + rev group ($p < .05$) but not the MK-801 sham + acq group. During the second, third, and fourth blocks, the MK-801 acq + rev group was significantly different from each of the other groups ($p < .01$), which did not differ among themselves. Overall, the subjects were more impaired during reversal than acquisition, and MK-801 caused a much greater impairment in reversal (acq + rev) than in acquisition (sham + acq). Like the analysis of acquisition, this analysis also suggested that the impairment due to MK-801 in Experiment 1 is specific to reversal learning and is not an artifact of sensitization to the second dose of MK-801.

Experiment 3

These experiments sought to determine whether the effects of MK-801 on reversal learning differed between two developmental ages, PND 21 and 30. These ages were chosen because they were 4 days younger or older, respectively, than the age investigated in Experiment 1. The design, procedure, and rationale for this experiment were the same as for Experiment 1 except that age was added as a variable and the 0.18 mg/kg dose of MK-801 was not used. Pups at each age were administered one of three doses of MK-801 (0.03, 0.06, or 0.10 mg/kg) or saline before both acquisition and reversal of a spatial discrimination in the T-maze. We avoided the highest dose used in Experiments 1 and 2 (0.18 mg/kg) because it marginally impaired acquisition and was accompanied by performance (motor) effects in some animals. The lower doses used in this study focused on reversal learning effects at doses that did not affect acquisition (or general performance).

Method

Subjects—Subjects were 96 Long Evans rats (47 male, 49 female) that were the offspring of 18 time-bred females. Male and female rats were obtained from Harlan breeders (Fredrick, Maryland), mated at the University of Delaware, and left undisturbed until birth. Except where noted, age determination, housing conditions, and weaning were as described in Experiment 1.

Pups trained on PND 21 were weaned from their mothers at PND 19; housed individually on heating pads as described in Pagani, Brown, and Stanton (2005); and subjected to the general acclimation procedures described in Experiment 1. Forty-eight pups were deprived at PND 19. The average weight at deprivation of this group was 49.0 ± 0.65 g (range = 32–50 g). Pups trained on PND 30 were weaned from their mothers at PND 21 and were housed with same-sex littermates with a continual supply of food and water until experimentation. Forty-eight pups were deprived at PND 28. The average weight at deprivation of this group was 85.7 ± 1.26 g (range = 68–100 g). One pup was excluded from this group because of inability to complete the task.

Apparatus—The apparatus was the same as described in Experiment 1.

Design—The design involved the between-subjects variables of age (PND 21 vs. 30) and treatment (0.03, 0.06, or 0.10 mg/kg MK-801 or saline vehicle). Other aspects of the design and procedure were the same as in Experiment 1. For acquisition and reversal training the design was a 2 (age) $\times$ 4 (treatment) $\times$ 2 (phase) $\times$ 4 (block) mixed factorial design.

Procedure—Pups were assigned to one of four groups at PND 21: saline ($n = 12$), 0.03 mg/kg MK-801 ($n = 12$), 0.06 mg/kg MK-801 ($n = 12$), and 0.10 mg/kg MK-801 ($n = 12$); or PND 30: saline ($n = 12$), 0.03 mg/kg MK-801 ($n = 12$), 0.06 mg/kg MK-801 ($n = 11$), and 0.10 mg/kg MK-801 ($n = 12$). For all pups, the procedure was deprivation, maze acclimation, training, and reversal. The procedures for these phases were identical to those described in Experiment 1.

Analysis—Dependent measures and data analysis were as described in Experiment 1 except that age was an additional variables in the ANOVA.

Results

The percentage correct data for each of four blocks of 12 trials during acquisition and reversal phases of training for this experiment are shown in Figure 3. Initial analyses indicated that there were no significant main effects of sex or nested variables, such as maze (1, 2, 3, or 4) or acquisition direction (left or right). Thus, data were pooled across these variables, and a 2 (age) $\times$ 4 (dose) $\times$ 2 (phase) $\times$ 4 (blocks) mixed design ANOVA was performed for acquisition and reversal.

At both test ages, subjects administered MK-801 performed similarly to saline controls during acquisition but were impaired in a dose-dependent manner during reversal learning. There were main effects of treatment, $F(3, 87) = 4.31, p < .01$; phase, $F(1, 87) = 87.64, p < .001$; and block, $F(3, 261) = 418.83, p < .001$. There were also significant interactions of Phase $\times$ Block, $F(3, 261) = 41.23, p < .001$; Treatment $\times$ Phase, $F(3, 87) = 7.78, p < .001$; Age $\times$ Phase, $F(1, 87) = 5.74, p < .05$; and Treatment $\times$ Block, $F(9, 261) = 3.41, p < .001$; as well as a marginally significant interaction of Treatment $\times$ Phase $\times$ Block, $F(9, 261) = 1.83, p < .065$, and Age $\times$ Phase $\times$ Block, $F(3, 261) = 2.22, p < .087$. The Phase $\times$ Block interaction was due to the subjects' making fewer correct choices at the outset of reversal than at the outset of acquisition but performing similarly near the end of each session. The interaction of Phase $\times$ Age is due to the greater change in performance between acquisition and reversal in the PND 21 group (77 vs. 55% correct, respectively; $p < .001$) than the PND 30 group (73 vs. 60%, respectively; $p < .001$). Newman–Keuls analysis demonstrated no significant differences ($p > .05$) between the PND 21 and 30 groups during either acquisition or reversal. The interaction of phase and treatment reflected the larger effect MK-801 had on reversal learning, as compared with acquisition. This is reflected in the Newman–Keuls tests, which revealed no significant differences between any of the groups during acquisition. However, during reversal, Newman–Keuls tests showed significant differences between the groups, whereby saline was significantly different from 0.06 mg/kg ($p < .01$) and 0.10 mg/kg ($p < .001$); 0.03 mg/kg was significantly different from 0.10 mg/kg ($p < .01$) and marginally different from 0.06 mg/kg ($p < .07$); and 0.06 mg/kg was marginally different from 0.10 mg/kg ($p < .07$). The Treatment $\times$ Block interaction was

driven largely by the effect of treatment during the reversal phase and reflected the fact that treatment effects were smaller in Blocks 1 and 4 and larger in Blocks 2 and 3. The marginal Treatment $\times$ Phase $\times$ Block interaction occurred because increasing doses of MK-801 impaired performance during reversal across an increasing number of blocks, relative to acquisition (which was unimpaired by MK-801).

In summary, MK-801 impaired reversal learning at both PND 21 and 30, suggesting that there is no developmental difference in the effects of MK-801 on reversal learning in weanling rats. MK-801 had a dose-dependent effect on reversal learning in weanling rats that was similar across the two ages studied in this experiment as well as in the PND 26 rats from Experiment 1.

Experiment 4

Experiment 4 was designed to further examine the specificity of MK-801 effects on reversal learning through drug administration only during reversal. In the previous experiments, MK-801 impaired reversal when it was given during both acquisition and reversal; thus, it is unclear during what stage of training the drug must be given in order to produce a reversal deficit. Experiment 4 was further designed to examine the role of state-dependent learning in the effects of MK-801 administered only during reversal. The state-dependency hypothesis predicts greater impairment when MK-801 is administered before both acquisition and reversal than before reversal only. This is because drug-related cues might create a stimulus change between acquisition and reversal and changing contextual cues between phases usually improves reversal performance (e.g., Pagani et al., 2005). Only the highest dose of MK-801 from the previous experiment was used in the present study. State-dependency effects were addressed by administering 0.10 mg/kg MK-801 before acquisition or reversal, both, or neither (saline vehicle). In addition, Experiment 4 was designed to determine whether these effects of MK-801 changed during development, through the inclusion of two other developmental ages, PND 21 and 30.

Method

Subjects—Subjects were 131 Long Evans rats (67 male, 64 female) that were the offspring of 22 time-bred females obtained, housed, and culled as described previously (Experiment 1). Forty-five pups were deprived at PND 24. The average weight at deprivation of this group was 55.9 ± 0.83 g (range = 41–68 g). Forty-three pups were deprived at PND 19, with an average weight at deprivation of 40.3 ± 0.55 g (range = 29–46 g). Pups trained on PND 30 were weaned from their mothers at PND 21 and were housed with same-sex littermates with a continual supply of food and water until experimentation. Forty-three pups were deprived at PND 28. The average weight at deprivation of this group was 79.6 ± 1.27 g (range = 54–95 grams). The pups were left undisturbed until PND 21, with the exception of pups that were trained on PND 21 (see below), who were weaned on PND 19. The remainder were only disturbed for routine cage changes as described in Experiment 1.

Pups trained on PND 21 were weaned from their mothers at PND 19 and were housed individually on heating pads as described in Experiment 3 and followed the general acclimation procedures described in Experiments 1 and 3. Additionally, following initial

deprivation at PND 19, pups were housed in cages that were heated on one side by an electric heating pad (GE Model E12107; General Electric, Fairfield, CT) set to the lowest setting.

This allowed the pups to self-regulate their temperature by seeking or avoiding the heated side of the cage.

Apparatus—The apparatus was the same as described in Experiment 1.

Design—The design involved the between-subjects variables of acquisition dose (saline vehicle or 0.10 mg/kg MK-801) and reversal dose (vehicle vs. MK-801). As in previous experiments, rats were trained in one direction during acquisition and reversed to the other direction in a subsequent reversal. Each acquisition and reversal session consisted of four blocks of 12 trials for a total of 48 trials. These sessions occurred in the morning and afternoon, starting about 6 hr apart. The rewarded goal arm, maze, sex, age, and dam were counterbalanced across subjects with each treatment and behavioral group. For acquisition and reversal training, the design was a 3 (age) $\times$ 2 (acquisition dose) $\times$ 2 (reversal dose) $\times$ 2 (phase) $\times$ 4 (block) mixed factorial design.

Procedure—For all pups, the procedure was deprivation, maze acclimation, training, and reversal. These phases are identical to those described in Experiment 1.

Forty-three rats were assigned to one of four groups at PND 21: vehicle–vehicle ($n = 11$), vehicle–MK-801 ($n = 12$), MK-801–vehicle ($n = 10$), or MK-801–MK-801 ($n = 10$). (Designations reflect the treatment during acquisition and reversal, respectively.) Forty-five rats were assigned to one of four groups at PND 26: vehicle–vehicle ($n = 13$), vehicle–MK-801 ($n = 11$), MK-801–vehicle ($n = 10$), or MK-801–MK-801 ($n = 11$). Forty-three rats were assigned to one of four groups at PND 30: vehicle–vehicle ($n = 11$), vehicle–MK-801 ($n = 10$), MK-801–vehicle ($n = 11$), or MK-801–MK-801 ($n = 11$). Specific procedures for drug administration and running sessions were as previously detailed in Experiment 1.

Analysis—Dependent measures and data analysis were as described in previous experiments. The variables examined in the ANOVA for this experiment were age (PND 21, 26, or 30), sex (male or female), acquisition dose (vehicle or MK-801), reversal dose (vehicle or MK-801), phase (acquisition or reversal), and block (1, 2, 3, or 4).

Results

The percentage correct data for each of four blocks of 12 trials during acquisition and reversal phases of training during this experiment are shown in Figure 4 (each panel shows a different age, and the fourth panel shows the data collapsed across ages). The results were similar for all three ages. All of the subjects, regardless of age or treatment, learned the discrimination during acquisition as shown by an increase in percentage correct across the four blocks of acquisition (1 through 4). During the first block of reversal, all of the groups had a lower percentage correct than occurred during any block of acquisition. On subsequent blocks, the groups administered saline (saline–saline and MK-801–saline) demonstrated reversal learning, with an increased percentage correct up to the same level as achieved

during acquisition. The two groups administered MK-801 during reversal (saline–MK-801 and MK-801–MK-801) were severely impaired during the first two blocks of reversal, making few correct choices. The MK-801–MK-801 group began to make more correct choices than the saline–MK-801 group starting in the third block and continuing until the end of the session. The saline–MK-801 group performed the most poorly during reversal and only made choices at chance level by the end of the reversal session. MK-801 did not have a state-dependent effect. MK-801 given only prior to reversal impaired performance more than did MK-801 given prior to both acquisition and reversal, although both of these groups were impaired relative to the groups administered saline before reversal.

Initial analyses indicated that there were no significant main effects of sex or nested variables such as maze (1, 2, 3, or 4) or acquisition direction (left or right). Thus, data were pooled across these variables, and a 3 (age) $\times$ 2 (acquisition treatment) $\times$ 2 (reversal treatment) $\times$ 2 (phase) $\times$ 4 (blocks) mixed-design ANOVA was performed for acquisition and reversal. There were main effects of reversal treatment, $F(1, 119) = 78.75, p < .001$; phase, $F(1, 119) = 181.60, p < .001$; and block, $F(3, 357) = 345.19, p < .001$. There was no main effect of age, but there was an interaction of Age $\times$ Phase $\times$ Block, $F(6, 357) = 2.55, p < .05$. There was no main effect of acquisition treatment, but there were interactions of Acquisition Treatment $\times$ Phase, $F(1, 119) = 10.66, p < .01$, and Acquisition Treatment $\times$ Reversal Treatment $\times$ Phase $\times$ Block, $F(3, 357) = 3.20, p < .05$. There were also interactions of Reversal Treatment $\times$ Phase, $F(1, 119) = 86.94, p < .001$; Reversal Treatment $\times$ Block, $F(3, 357) = 8.22, p < .001$; and Reversal Treatment $\times$ Phase $\times$ Block, $F(3, 357) = 4.30, p < .01$. Newman–Keuls analysis of the Acquisition Treatment $\times$ Reversal Treatment $\times$ Phase $\times$ Block interaction failed to reveal any difference among treatment groups during acquisition. During reversal, the saline–saline and MK-801–saline groups differed only during Block 1 ($p < .028$). Thus, rats receiving saline during reversal performed similarly, regardless of whether they received saline or MK-801 during acquisition. The MK-801–MK-801 and saline–MK-801 groups failed to differ in the first two blocks of reversal but differed significantly in Blocks 3 and 4 ($ps < .003$). Thus, reversal learning was more impaired in rats receiving MK-801 during reversal only than in rats receiving the drug during both acquisition and reversal (the opposite effect predicted by state-dependent learning to drug-related cues). Finally, the saline–saline group differed from the saline–MK-801 and MK-801–MK-801 groups on all reversal blocks ($p < .001$) except Block 1, whereas the MK-801–saline group differed from these two groups on all reversal blocks ($p < .001$). These data indicate that impairment of reversal by MK-801 reflects an action of the drug during the reversal phase of training. These results also negate the state-dependency hypothesis, which predicts greater impairment when drug treatments are the same during both acquisition and reversal than when they change across these phases because the stimulus change is expected to improve reversal performance. The transition from MK-801 to saline across phases modestly improved reversal only in Block 1, whereas the transition from saline to MK-801 impaired reversal in Blocks 3 and 4. This pattern of results is not easily interpreted in terms of state-dependent learning to drug-related cues. The other main conclusions from this experiment were that spatial reversal is more sensitive to NMDA receptor antagonism than acquisition and that sensitivity of spatial reversal learning to this dose of MK-801 does not change between PND 21 and PND 30.

Analysis of Reversal Errors—Previous studies have shown that blockade in different brain regions affects the types of errors made during reversal learning. For example, blockade of NMDA receptors and muscarinic cholinergic receptors in the dorsomedial striatum or inactivation of this region with tetracaine leads to a deficit in reversal learning that is specific to an inability to acquire a new strategy (“regressive errors”) rather than perseveration of the previously learned strategy (Palencia & Ragozzino, 2004; Ragozzino, Jih, & Tzavos, 2002; Ragozzino, Ragozzino, Mizumori, & Kesner, 2002). Damage to the orbital prefrontal cortex impairs perseverative responding more than regressive responding (Dias, Robbins, & Roberts, 1997). To gather indirect evidence regarding what structures might be targeted by systemic MK-801, we divided the errors made during reversal learning into perseverative and regressive errors (explained more fully below). Additional measures were analyzed during the reversal phase to determine whether MK-801 affected either trials to criterion (10 correct responses in 12 trials) or total errors. To maximize statistical power of this analysis and make the results more indicative of the study as a whole, we pooled data from subjects run in Experiments 1, 2, and 4 that met certain criteria. The subjects had to be the same age (PND 26) and be administered the same drug for both acquisition and reversal (e.g., saline–saline or MK-801–MK-801). These data yielded 4 new groups: saline ($n = 35$), 0.06 mg/kg MK-801 ($n = 11$), 0.10 mg/kg MK-801 ($n = 23$), and 0.18 mg/kg MK-801 ($n = 20$). These groups were analyzed for trials to criterion and perseverative, regressive, and total errors. We conducted planned comparisons, using t tests to examine how each dose differed from saline or the other MK-801 doses.

Trials to criterion were examined for information on the differential speed of learning among the groups by calculating the number of trials required for each subject to perform better than chance, here defined as at least 10 correct choices in 12 successive trials. If a subject did not meet this criterion, it was assigned the maximum number of trials for the session (48). The differential speed of learning during the reversal phase was analyzed with trials to criterion (Figure 5, top left panel). We performed a one-way ANOVA of the trials to criterion data by MK-801 dose. There was a main effect of MK-801 dose, $F(3, 85) = 12.99$, $p < .001$. Planned comparisons conducted with t tests revealed that saline was significantly different from the 0.1 and 0.18 mg/kg dose of MK-801 ($ps < .001$) but was only marginally significantly different from the lowest dose, 0.06 mg/kg ($p < .09$). The highest dose of MK-801 was significantly different from all of the other doses ($ps < .05$), and the middle two MK-801 doses (0.06 and 0.1 mg/kg) did not differ from each other.

Perseverative errors and regressive errors were defined according to criteria established in previous studies (Dias & Aggleton, 2000; Hunt & Aggleton, 1998; Palencia & Ragozzino, 2004; Ragozzino, Detrick, & Kesner, 1999; Ragozzino, Jih, & Tzavos, 2002). Perseverative errors were operationally defined as incorrect choices three or more times in consecutive blocks of four trials each. Once the subject made fewer than three errors in a block, all subsequent errors were counted as regressive errors. Thus, perseverative errors measure the ability of the subject to shift away from the previously reinforced choice, and regressive errors measure the ability of the subject to subsequently learn the new correct choice. Total errors were the sum of the perseverative and regressive errors.

We performed a one-way ANOVA of perseverative errors (Figure 5, bottom left panel). There was a main effect of MK-801 dose, $F(3, 85) = 3.61, p < .05$. Planned comparisons conducted with t tests revealed that saline was significantly different from the 0.1 and 0.18 mg/kg doses of MK-801 ($ps < .05$) but was not different from the lowest dose (0.06 mg/kg). The 0.06 and 0.1 mg/kg MK-801 doses were also significantly different from each other ($p < .05$), but 0.18 mg/kg MK-801 did not differ from the lower MK-801 doses.

A one-way ANOVA was performed on regressive errors (Figure 5, bottom right panel). There was a main effect of MK-801 dose, $F(3, 85) = 3.61, p < .05$. Planned comparisons conducted with t tests revealed that saline was significantly different from the 0.06 and 0.18 mg/kg doses of MK-801 ($ps < .01$) and almost significantly different from the middle dose, 0.10 mg/kg ($p < .07$). None of the MK-801 doses were different from each other. MK-801 dose dependently increased both perseverative and regressive errors during the reversal phase, suggesting that there are deficits in both processes, perseveration of the previously learned strategy and an ability to learn the new discrimination. Perseveration was significantly increased in the pups dosed with the higher doses of MK-801. An increase in perseveration is clearly one component of the behavioral effects of MK-801 during reversal, and this may reflect targeting of orbitofrontal cortex (see General Discussion). However, regressive errors were clearly another component of the impaired reversal learning produced by MK-801. This suggests that orbitofrontal targeting is not sufficient to account for the entire effects of systemic MK-801 on this reversal task.

The overall effect of MK-801 during the reversal phase was analyzed with total errors (Figure 5, top right panel). We performed a one-way ANOVA of the total errors data by MK-801 dose. There was a main effect of MK-801 dose, $F(3, 85) = 19.32, p < .001$. Planned comparisons conducted with t tests revealed that saline was significantly different from all of the MK-801 doses ($ps < .05$). The highest dose of MK-801 (0.18 mg/kg) was significantly different from all of the other doses ($ps < .05$), and the middle two MK-801 doses (0.06 and 0.1 mg/kg) did not differ from each other. These results are very similar to the trials-to-criterion data.

General Discussion

Four experiments in developing rats examined the effects of NMDA receptor antagonism on reversal learning of a spatial discrimination. Experiment 1 determined which doses of MK-801 disrupted reversal learning of spatial discrimination in PND 26 rats without affecting acquisition. There was a dose-dependent effect of MK-801, whereby doses of 0.10 and 0.18 mg/kg, but not 0.06 mg/kg, significantly disrupted reversal learning. The highest dose tested in this experiment (0.18 mg/kg) also tended to disrupt acquisition of spatial discrimination, though this may have reflected a “performance effect” (see below). Experiment 2 showed that the effects of MK-801 on reversal learning could not be attributed to sensitization to a second dose of MK-801. MK-801 impaired reversal to a much greater extent than acquisition even when acquisition and reversal were both tested after the second administration of MK-801. Experiment 3 extended the design of the first experiment to other developmental ages, PND 21 and 30. MK-801 given during acquisition and reversal again selectively disrupted reversal learning in a dose-dependent manner, but there were no

differences in the effects of MK-801 across these ages. Experiment 4 determined that the effects of MK-801 on reversal learning reflect its actions during reversal rather than acquisition and that state-dependent learning effects are not seen with MK-801 at any of the ages tested: PND 21, 26, or 30.

Studies in adult rats and mice have found a similar effect of NMDA receptor antagonists on reversal learning without affecting acquisition, as shown in weanling rats in the current set of experiments. MK-801 had no effect on acquisition but impaired reversal learning in rats in the Y-maze (Murray et al., 1995) and in mice in the water T-maze (Bardgett et al., 2003), whereas D(-)-2-amino-5-phosphonopentanoic acid (AP-5), a competitive NMDA receptor antagonist, administered directly into the dorsomedial striatum of rats impaired reversal learning in a modified cross maze without affecting acquisition (Palencia & Ragozzino, 2004). One study in adult rats also used several doses of MK-801 to examine acquisition and reversal of a two-lever discrimination task (van der Meulen et al., 2003). The results from this study also support a dose-dependent effect of MK-801 whereby the low doses did not impair acquisition but did impair reversal and the highest dose used impaired acquisition. This is very similar to the results reported here in weanling rats, where the highest dose of MK-801 used impaired acquisition, but the lower doses only impaired reversal. When impairments in both acquisition and reversal are found, it is difficult to unequivocally conclude that the drug is affecting learning rather than performance. In our study, we observed modest motor effects in some rats treated with the 0.18 mg/kg dose. It is therefore possible that poor performance during both acquisition and reversal at this dose is not the result of an effect of MK-801 specifically on learning. In contrast, it is unlikely that doses of MK-801 that selectively impair reversal are disrupting general performance rather than cognitive processes.

The greater sensitivity of reversal to MK-801 may reflect the fact that acquisition and reversal learning engage different psychological processes. For example, there is associative interference during reversal learning, whereby the previously learned association and the new (reversed) association are competing for expression (Pagani et al., 2005). When there are conflicting associations during reversal learning, contextual cues become more important determinants of behavior such that what is learned second becomes “tied” to the context (Bouton, 1993). This has been shown in experiments where manipulations of the context between acquisition and reversal or extinction have enhanced reversal or extinction learning in both weanling rats (Moye, Brasser, Palmer, & Zeisset, 1992; Pagani et al., 2005) and adults (McDonald, King, & Hong, 2001). MK-801 has been shown to impair contextual learning (Bardgett et al., 2003; Csernansky et al., 2005; Gould, McCarthy, & Keith, 2002). There were no contextual changes in the current experiment, but MK-801 may have impaired performance during the reversal task by interfering with contextual learning, which appears to be more important during reversal learning than during acquisition. This could be further tested by changing the contextual cues for each maze in MK-801- or vehicle-treated rats. MK-801 might attenuate the effects of context shifts on reversal learning performance.

The present study confirms and extends previous demonstrations that MK-801 can impair learning and memory in developing rats (Griesbach et al., 1998; Highfield et al., 1996). Griesbach et al. (1998) reported that acquisition of odor discrimination in rats of postnatal

age 22 to 28 days is impaired by intraperitoneal administration of 0.05 mg/kg MK-801. Reversal learning was impaired only when the drug was administered before reversal and not when it was administered during both the acquisition and reversal learning phases. This contrasts with the current findings, in which reversal was impaired under both dosing regimens and acquisition was not impaired at comparable doses of MK-801. Griesbach et al (1998, Experiment 3) ruled out performance effects of MK-801 by showing that the drug did not disrupt performance of a previously learned odor discrimination. Odor discrimination is a more difficult task for weanling rats to learn, as shown by the increased number of sessions necessary to meet criterion for learning. Differences in task difficulty between the olfactory versus spatial discrimination may account for the different effects of MK-801 in the Griesbach et al. study relative to the present one. Another study examining the effects of MK-801 on a nonspatial working memory task, patterned single alternation, in preweanling rats showed that MK-801 produced larger impairments as the task became more difficult (Highfield et al., 1996). Our study did not manipulate task difficulty during acquisition, although reversal learning is thought to be more difficult or to involve more learning processes than acquisition (McAlonan & Brown, 2003; White, 2004). Another significant difference between the present study and Griesbach et al.'s (1998) study is that in Griesbach et al., MK-801 was administered three times a day at 4-hr intervals for several days during each training phase. This led the authors to suggest that tolerance to the effects of MK-801 may have accounted for the lack of impairment in reversal learning when the drug was administered before both acquisition and reversal in their odor discrimination task (such tolerance was demonstrated previously in adult rats; Wessinger, 1994). The present study explicitly excluded tolerance and sensitization to MK-801 as a factor that could account for differences in drug effects across training phases or sessions (Experiment 2). However, there may have been some tolerance to the nonspecific effects of MK-801 that affected performance. In Experiment 4, subjects in the saline–MK-801 group performed significantly worse than those in the MK-801–MK-801 group. Both groups were administered MK-801 prior to reversal, but the saline–MK-801 group made significantly more errors. It may be that the rats given MK-801 during both phases had a chance to habituate to nonspecific effects of the drug, which could have contributed to the performance in the saline–MK-801 group.

Finally, the present study involved several doses of MK-801 and showed that impairment of reversal learning is dose dependent, whereas previous studies only included a single 0.05 mg/kg dose of MK-801. However, the present study joins these previous studies with developing rats (Griesbach et al., 1998; Highfield et al., 1996) to suggest that the role of the NMDA receptor system is well developed by the periweanling period. In adult rats, MK-801 impaired reversal learning at doses up to 0.075 mg/kg (Murray et al., 1995), but when the dose was increased to 0.10 mg/kg MK-801, there was also an impairment of acquisition (van der Meulen et al., 2003). Weanling rats showed a similar pattern at slightly higher doses of MK-801 in the current study. MK-801 in doses up to 0.10 mg/kg impaired reversal learning without affecting acquisition, and a dose of 0.18 mg/kg MK-801 impaired acquisition marginally in Experiment 1 and significantly in Experiment 2. This suggests that sensitivity to MK-801 in spatial reversal may change between PND 30 and adulthood. However, there were differences in the designs of each experiment, and it would be useful to run a direct

comparison of weanling vs. adult rats under the same conditions before reaching this conclusion. Our failure to find any age-related changes in MK-801 sensitivity between PND 21 and 30 suggest that the NMDA receptor system is relatively mature by the end of the 3rd week of life in the rat.

In this set of experiments, acquisition of a spatial discrimination appears to be a non-NMDA-mediated phenomenon, at least at the lower doses of MK-801 that impaired reversal learning but not acquisition. NMDA receptors are found throughout the brain, with the highest concentrations of [³H]-MK-801 binding in the frontal cortex, striatum, entorhinal cortex, CA1, and the dentate gyrus, and lower levels found in the cerebellar granule cell layer (Porter & Greenamyre, 1995). The hippocampus, with the high concentrations of NMDA receptors, is thought to underlie many forms of learning and is thought to be very important for spatial learning. However, there is some evidence that the hippocampus is not necessary for very simple spatial discrimination, though it may be necessary for reversal (Murray & Ridley, 1999; Oliveira, Bueno, Pomarico, & Gugliano, 1997). Lesions to the hippocampus did not impair acquisition or reversal of a spatial discrimination task in the Y-maze (Murray & Ridley, 1999); however, in the elevated T-maze, hippocampal lesions impaired reversal learning without affecting acquisition (Oliveira et al., 1997). However, regions of the striatum and prefrontal cortex do appear to be necessary for simple spatial reversal learning. For example, inactivation of the dorsomedial striatum impaired reversal learning without affecting acquisition (Ragozzino & Choi, 2004; Ragozzino, Jih, & Tzavos, 2002). This impairment seems to reflect primarily an increase in regressive errors — slower reversal learning after the initial perseverative phase (Palencia & Ragozzino, 2004). Different regions of the prefrontal cortex also appear to be important in reversal learning, as lesions of the orbital prefrontal cortex impair reversal learning (Kim & Ragozzino, 2005; Kolb & Nanneman, 1978; McAlonan & Brown, 2003) but lesions of the medial prefrontal cortex do not (Salazar, White, Lacroix, Feldon, & White, 2004). Orbital prefrontal inactivation appears to impair reversal learning by disproportionately increasing perseverative errors relative to regressive errors (Kim & Ragozzino, 2005). In the current study, MK-801 impaired reversal learning in a manner that reflected increases in both perseverative and regressive errors (Figure 5). This suggests that in the present study, systemic MK-801 targeted both the striatum and frontal cortex and altered plasticity in both brain regions, causing impaired reversal learning of T-maze discrimination.

Spatial reversal learning and NMDA antagonism are relevant to two different clinical disorders, autism and schizophrenia. Reversal learning is impaired in autism (Coldren & Halloran, 2003), and there is some evidence for glutamate dysfunction in autism (Carlsson, 1998). Schizophrenic patients do poorly on tasks thought to be differentially sensitive to atrophic lesions of the frontal and temporal cortices (Kolb & Whishaw, 1983), the same areas of the brain thought to underlie spatial reversal learning. Hypofunction of the NMDA receptor is postulated to underlie the pathophysiology of schizophrenia (Laruelle, Frankle, Narendran, Kegeles, & Abi-Dargham, 2005), which is modeled in rats by MK-801 administration (Csernansky et al., 2005). Therefore, understanding the NMDA receptor contribution to reversal learning may contribute to the development of animal models that can help provide a better understanding of these clinical disorders. For example, the present demonstration that NMDA receptor antagonism disrupts reversal learning during

development supports the use of this task in developmental analysis of rodent models of autism.

References

- Bardgett ME, Boeckman R, Krochmal D, Fernando H, Ahrens R, Csernansky JG. NMDA receptor blockade and hippocampal neuronal loss impair fear conditioning and position habit reversal in C57Bl/6 mice. *Brain Research Bulletin*. 2003; 60:131–142. [PubMed: 12725901]
- Bear MF, Rittenhouse CD. Molecular basis for induction of ocular dominance plasticity. *Journal of Neurobiology*. 1999; 41:83–91. [PubMed: 10504195]
- Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *Journal of Physiology*. 1973; 232:331–356. [PubMed: 4727084]
- Bouton ME. Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. *Psychological Bulletin*. 1993; 114:80–99. [PubMed: 8346330]
- Carlsson ML. Hypothesis: Is infantile autism a hypoglutamatergic disorder? Relevance of glutamate–serotonin interactions for pharmacotherapy. *Journal of Neural Transmission*. 1998; 105:525–535. [PubMed: 9720980]
- Coldren JT, Halloran C. Spatial reversal as a measure of executive functioning in children with autism. *Journal of Genetic Psychology*. 2003; 164:29–41. [PubMed: 12693742]
- Collingridge GL, Kehl SJ, McLennan H. The antagonism of amino acid-induced excitations of rat hippocampal CA1 neurones in vitro. *Journal of Physiology*. 1983; 334:19–31. [PubMed: 6134823]
- Colwell CS, Cepeda C, Crawford C, Levine MS. Post-natal development of glutamate receptor-mediated responses in the neostriatum. *Developmental Neuroscience*. 1998; 20:154–163. [PubMed: 9691190]
- Csernansky JG, Martin M, Shah R, Bertchume A, Colvin J, Dong H. Cholinesterase inhibitors ameliorate behavioral deficits induced by MK-801 in mice. *Neuropsychopharmacology*. 2005; 30:2135–2143. [PubMed: 15956997]
- Dias R, Aggleton JP. Effects of selective excitotoxic pre-frontal lesions on acquisition of nonmatching- and matching-to-place in the T-maze in the rat: Differential involvement of the prelimbic–infralimbic and anterior cingulate cortices in providing behavioural flexibility. *European Journal of Neuroscience*. 2000; 12:4457–4466. [PubMed: 11122356]
- Dias R, Robbins TW, Roberts AC. Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: Restriction to novel situations and independence from “on-line” processing. *Journal of Neuroscience*. 1997; 17:9285–9297. [PubMed: 9364074]
- Douglas RM, Goddard GV. Long-term potentiation of the perforant path–granule cell synapse in the rat hippocampus. *Brain Research*. 1975; 86:205–215. [PubMed: 163667]
- Dudek SM, Bear MF. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *Journal of Neuroscience*. 1993; 13:2910–2918. [PubMed: 8331379]
- Freeman JH Jr, Stanton ME. Fimbria-fornix transections disrupt the ontogeny of delayed alternation but not position discrimination in the rat. *Behavioral Neuroscience*. 1991; 105(3):386–395. [PubMed: 1863360]
- Gould TJ, McCarthy MM, Keith RA. MK-801 disrupts acquisition of contextual fear conditioning but enhances memory consolidation of cued fear conditioning. *Behavioural Pharmacology*. 2002; 13:287–294. [PubMed: 12218509]
- Griesbach GS, Hu D, Amsel A. Effects of MK-801 on vicarious trial-and-error and reversal of olfactory discrimination learning in weanling rats. *Behavioural Brain Research*. 1998; 97:29–38. [PubMed: 9867228]
- Highfield DA, Nixon K, Amsel A. The NMDA antagonist MK-801 affects nonspatial learning in preweanling rats. *Behavioral Neuroscience*. 1996; 110:300–304. [PubMed: 8731056]
- Hunt PR, Aggleton JP. Neurotoxic lesions of the dorsomedial thalamus impair the acquisition but not the performance of delayed matching to place by rats: A deficit in shifting response rules. *Journal of Neuroscience*. 1998; 18:10045–10052. [PubMed: 9822759]

- Kim J, Ragozzino ME. The involvement of the orbitofrontal cortex in learning under changing task contingencies. *Neurobiology of Learning and Memory*. 2005; 83:125–133. [PubMed: 15721796]
- Kolb B, Nonneman AJ. Sparing of function in rats with early prefrontal cortex lesions. *Brain Research*. 1978; 151:135–148. [PubMed: 678999]
- Kolb B, Whishaw IQ. Performance of schizophrenic patients on tests sensitive to left or right frontal, temporal, or parietal function in neurological patients. *Journal of Nervous and Mental Disease*. 1983; 171:435–443. [PubMed: 6864202]
- Laruelle M, Frankle WG, Narendran R, Kegeles LS, Abi-Dargham A. Mechanism of action of antipsychotic drugs: From dopamine D(2) receptor antagonism to glutamate NMDA facilitation. *Clinical Therapeutics*. 2005; 27A:S16–S24. [PubMed: 16198197]
- Lincoln J, Coopersmith R, Harris EW, Cotman CW, Leon M. NMDA receptor activation and early olfactory learning. *Brain Research*. 1988; 467:309–312. [PubMed: 2897868]
- Lynch G, Baudry M. The biochemistry of memory: A new and specific hypothesis. *Science*. 1984; 224:1057–1063. [PubMed: 6144182]
- McAlonan K, Brown VJ. Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behavioural Brain Research*. 2003; 146:97–103. [PubMed: 14643463]
- McDonald RJ, King AL, Hong NS. Context-specific interference on reversal learning of a stimulus–response habit. *Behavioural Brain Research*. 2001; 121:149–165. [PubMed: 11275292]
- Moye TB, Brasser SM, Palmer L, Zeisset C. Contextual control of conflicting associations in the developing rat. *Developmental Psychobiology*. 1992; 25:151–164. [PubMed: 1618368]
- Murray TK, Ridley RM. The effect of excitotoxic hippocampal lesions on simple and conditional discrimination learning in the rat. *Behavioural Brain Research*. 1999; 99:103–113. [PubMed: 10512577]
- Murray TK, Ridley RM, Snape MF, Cross AJ. The effect of dizocilpine (MK-801) on spatial and visual discrimination tasks in the rat. *Behavioural Pharmacology*. 1995; 6:540–549. [PubMed: 11224361]
- Nakazawa K, McHugh TJ, Wilson MA, Tonegawa S. NMDA receptors, place cells and hippocampal spatial memory. *Nature Reviews Neuroscience*. 2004; 5:361–372.
- Oliveira MG, Bueno OF, Pomarico AC, Gugliano EB. Strategies used by hippocampal- and caudate-putamen-lesioned rats in a learning task. *Neurobiology of Learning and Memory*. 1997; 68:32–41. [PubMed: 9195587]
- Pagani JH, Brown KL, Stanton ME. Contextual modulation of spatial discrimination reversal in developing rats. *Developmental Psychobiology*. 2005; 46:36–46. [PubMed: 15690387]
- Palencia CA, Ragozzino ME. The influence of NMDA receptors in the dorsomedial striatum on response reversal learning. *Neurobiology of Learning and Memory*. 2004; 82:81–89. [PubMed: 15341793]
- Porter RH, Greenamyre JT. Regional variations in the pharmacology of NMDA receptor channel blockers: Implications for therapeutic potential. *Journal of Neurochemistry*. 1995; 64:614–623. [PubMed: 7530291]
- Quinlan EM, Olstein DH, Bear MF. Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. *Proceedings of the National Academy of Sciences USA*. 1999; 96:12876–12880.
- Ragozzino ME, Choi D. Dynamic changes in acetylcholine output in the medial striatum during place reversal learning. *Learning and Memory*. 2004; 11:70–77. [PubMed: 14747519]
- Ragozzino ME, Detrick S, Kesner RP. Involvement of the prelimbic–infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. *Journal of Neuroscience*. 1999; 19:4585–4594. [PubMed: 10341256]
- Ragozzino ME, Jih J, Tzavos A. Involvement of the dorsomedial striatum in behavioral flexibility: Role of muscarinic cholinergic receptors. *Brain Research*. 2002; 953:205–214. [PubMed: 12384254]
- Ragozzino ME, Ragozzino KE, Mizumori SJ, Kesner RP. Role of the dorsomedial striatum in behavioral flexibility for response and visual cue discrimination learning. *Behavioral Neuroscience*. 2002; 116:105–115. [PubMed: 11898801]

- Salazar RF, White W, Lacroix L, Feldon J, White IM. NMDA lesions in the medial prefrontal cortex impair the ability to inhibit responses during reversal of a simple spatial discrimination. *Behavioural Brain Research*. 2004; 152:413–424. [PubMed: 15196810]
- Simon DK, Prusky GT, O'Leary DD, Constantine-Paton M. N-methyl-D-aspartate receptor antagonists disrupt the formation of a mammalian neural map. *Proceedings of the National Academy of Sciences USA*. 1992; 89:10593–10597.
- Teyler TJ. Comparative aspects of hippocampal and neocortical long-term potentiation. *Journal of Neuroscience Methods*. 1989; 28:101–108. [PubMed: 2542697]
- van der Meulen JA, Bilbija L, Joosten RN, de Bruin JP, Feenstra MG. The NMDA-receptor antagonist MK-801 selectively disrupts reversal learning in rats. *NeuroReport*. 2003; 14:2225–2228. [PubMed: 14625452]
- Watson DJ, Sullivan JR, Frank JG, Stanton ME. Serial reversal learning of position discrimination in developing rats. *Developmental Psychobiology*. 2006; 48:79–94. [PubMed: 16381028]
- Wessinger WD. Tolerance to and dependence on MK-801 (dizocilpine) in rats. *Pharmacology, Biochemistry and Behavior*. 1994; 49:1049–1056.
- White NM. The role of stimulus ambiguity and movement in spatial navigation: A multiple memory systems analysis of location discrimination. *Neurobiology of Learning and Memory*. 2004; 82:216–229. [PubMed: 15464405]

Figure 1.

Mean ($\pm$ standard error of the mean) percentage correct responses for the four dizocilpine maleate (MK-801) dose groups in Experiment 1 as a function of 12-trial blocks of training administered on postnatal day (PND) 26. For each of the four groups, the dose of MK-801 was saline (closed circle), 0.06 mg/kg (open triangle), 0.10 mg/kg (closed square), or 0.18 mg/kg (open diamond). Dashed horizontal line at 50% indicates chance performance. MK-801 was administered before both acquisition (A) and reversal (R).

Figure 2.

Mean ($\pm$ standard error of the mean) percentage correct responses for the four groups in Experiment 3 as a function of treatment, training session, and 12-trial blocks administered on postnatal day (PND) 26. Either dizocilpine maleate MK-801 (0.18 mg/kg) or saline was administered before the morning session (acquisition or “sham”) and afternoon session (acquisition [acq] or reversal [rev]). The resulting groups were saline acq + rev (closed circle), MK-801 acq + rev (closed square), saline sham + acq (open circle), and MK-801 sham + acq (open square). See text for further explanation. Dashed horizontal line at 50% indicates chance performance.

Figure 3.

Mean ($\pm$ standard error of the mean) percentage correct responses for the four dizocilpine maleate (MK-801) dose groups in Experiment 2 as a function of 12-trial blocks of training administered on postnatal days (PNDs) 21 and 30. For each of the four groups, the dose of MK-801 was saline (closed circle), 0.03 mg/kg (dotted circle), 0.06 mg/kg (open triangle), or 0.10 mg/kg (closed square). Dashed horizontal line at 50% indicates chance performance. MK-801 was administered before both acquisition (A) and reversal (R).

Figure 4.

Mean ($\pm$ standard error of the mean) percentage correct responses for the four groups in Experiment 4 as a function of age (Postnatal day [PND] 21, 26, or 30, or all ages combined), treatment (0.10 mg/kg dizocilpine maleate [MK-801] or saline), training phase (acquisition vs. reversal), and 12-trial blocks. At each age, there were four groups given either MK-801 or saline before either the acquisition (A) or reversal (R) session. The groups were saline–saline (Sal–Sal; closed circle), saline–MK-801 (Sal–MK; open circle), MK-801–saline (MK–Sal; open square), or MK-801–MK-801 (MK–MK; closed square). Dashed horizontal line at 50% indicates chance performance.

Figure 5.

Analysis of trials to criterion and error types during reversal for postnatal day 26 rats as a function of dose of dizocilpine maleate (MK-801); data were pooled from Experiments 1, 2, and 4. Mean ($\pm$ standard error of the mean) trials to criterion (A), total errors (B), perseverative errors (C), and regressive errors (D). See text for further explanation.