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Behavioral effects of MK-801 in the rat

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Abstract. Several experiments were conducted to study the effects of the noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, on learning and memory in the rat. Rats displayed impaired performance on several sensorimotor tests and appeared grossly intoxicated when treated IP with 0.2 mg/kg MK-801, but not when treated with lower doses (0.05 or 0.1 mg/kg). Postacquisition performance on two spatial learning tasks involving working memory protocols (reinforced alternation and radial arm maze) was impaired by MK-801 at intoxicating doses ($\geq$0.2 mg/kg) but not at lower doses (0.05 or 0.1 mg/kg). Using a position habit reversal task, we found that rats could learn to reverse a position habit while under the influence of a nonintoxicating dose of MK-801 (0.1 mg/kg), but when tested on the following day performed as if they did not recall what they had learned. Thus, acute administration of a nonintoxicating dose of MK-801 disrupts the retention of new information learned under the influence of the drug but does not interfere with the performance of tasks that are well learned before the drug is administered. Whether the performance deficits on the spatial learning tasks observed only following intoxicating doses of MK-801 reflect an effect on memory is not clear.

Key words: MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate] – N-methyl-D-aspartate receptor antagonists – Learning and memory – Sensorimotor function

Interest in a possible role for the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid (EAA) receptor in memory has been stimulated by evidence that NMDA receptors are involved in the mediation of long term potentiation (LTP), a phenomenon hypothetically linked to memory formation (Lynch and Baudry 1984). Involvement of NMDA receptors in LTP was first documented in studies showing that either competitive (Collingridge et al. 1983) or noncompetitive (Stringer et al. 1983) NMDA antagonists block induction of LTP in the CA1 region of the rat hippocampus. More recently, it was reported that chronic infusion of the competitive NMDA antagonist, 2-amino-5-phosphonopentanoate (AP5), into the lateral ventricle of rat brain interfered with induction of LTP in the in vivo hippocampus and impaired performance on a spatial learning task (Morris et al. 1986) and on a nonspatial operant learning task (Tonkiss et al. 1988). Systemic injection of noncompetitive NMDA antagonists such as phencyclidine (PCP) and the closely related PCP receptor ligand, MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate], also reportedly produce performance deficits on learning and memory tasks (Kesner et al. 1983; Handelmann et al. 1987; Benvenga and Spaulding 1988; Danysz et al. 1988; Mondadori et al. 1988; Staubli et al. 1989). The latter agents do not interact directly with the NMDA receptor but rather with PCP receptors which are coupled to the NMDA receptor ion channel. Thus, a blocking action directed either toward the NMDA receptor or its ion channel may be effective in interfering with learning/memory processes.

The use of competitive NMDA antagonists, such as AP5, to study the role of NMDA receptors in memory is fraught with significant problems. Because these agents do not readily penetrate blood-brain barriers, they must be infused directly into the brain which introduces the invasiveness of the method as a potential confounding variable. The ease with which noncompetitive NMDA antagonists penetrate blood-brain barriers recommends these agents for exploring the role of NMDA receptors in memory. However, interpretation of studies using either competitive or noncompetitive NMDA antagonists is complicated by the fact that memory impairment is but one of several possible mechanisms by which

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such agents can disrupt performance on learning tasks. Indeed, these agents can induce disturbances in both sensory and motor functions, but most studies have not attempted to delineate the role of such disturbances in the animals' performance in learning tasks.

In the present study, we evaluated the effects of systemically administered MK-801 on the performance of adult rats on several working (trial-dependent) memory tasks (reinforced alternation and several radial arm maze protocols) and on a one-trial memory retention test involving the reversal of a position habit. In addition, to help identify factors other than memory impairment by which MK-801 might influence performance, rats were treated with various doses of MK-801 and tested on a battery of sensorimotor tasks.

Materials and methods

Male (Sprague Dawley) rats served as subjects in all of the experiments. They were housed in pairs in plastic cages containing woodchip bedding and were maintained on a 12-h light/dark cycle. In experiments 1, 2, and 4, rats were reduced to and maintained at approximately 85–90% of their free-feeding body weight by limiting food (wet mash) access to 1 h per day throughout the duration of the experiments. Behavioral testing was initiated after 1 week of the restricted access to food. Water was available at all times. Specific ages of the rats are noted below for each experiment.

Experiment 1: Effects of MK-801 on reinforced alternation

Training

Ten, 75-day-old rats were trained to choose alternate sides of a standard T-maze (maze described in Wozniak et al. 1989) on successive trials in order to receive a food reward. On the first trial of a session, a rat was reinforced for choosing either side of the T-maze. On subsequent trials, the rat was rewarded only for choosing the side opposite to the one in which it last received reinforcement. A response was defined when a rat extended its body past the choice point and placed all four paws within a wing. The guillotine door was lowered following a response, in order to prevent retracing. Olfactory cues were balanced by placing a food cup filled with wet mash at the end of each wing and both wings were washed with a scented detergent after every trial. An opaque Plexiglas barrier prevented access to each cup and was raised to allow the rat to eat for 20 s following a correct response. Following an incorrect response, the rat was confined to the chosen wing for 20 s without being rewarded. Rats were run in a random order for ten (massed) trials per day, 7 days a week for 30 consecutive days. A 30-s intertrial interval (ITI) was used during training. An acquisition criterion of at least nine correct responses out of ten for 4 consecutive days was used to evaluate how well the animals had learned the task before drug testing began.

Drug testing

MK-801 was dissolved in distilled water and adjusted to a neutral pH (7.3 ± 0.1) with NaOH. Drug challenge procedures commenced on day 31 and were carried out on every third day thereafter up to day 40. On day 31, five rats received injections of 0.05 mg/kg MK-801 (injection volumes $\simeq 0.5$ ml) and five received saline. On day 34, the treatment conditions were reversed. The same protocols were used on days 37 and 40 except that 0.1 mg/kg MK-801 was used. The treatment status of the animals for the first challenge (day 31) was determined randomly and the treatment (MK-801 versus saline) was alternated over the first four challenges (days 30 and 31 and days 37 and 40). On day 46, all rats were challenged with 0.5 mg/kg MK-801. On days 50 and 51, the "memory load" of the task was increased by lengthening the ITI from 30 s to 2 min and rats were injected with either saline or 0.1 mg/kg MK-801 on the successive challenges. Injections were given 20 min before each test.

Statistical analyses

A two-way ANOVA (Wilkinson 1987/SYSTAT) consisting of two within-subjects variables, treatment (MK-801 versus saline) and dose (0.05 versus 0.1 mg/kg MK-801), was used to analyze the working memory data (number of correct responses out of ten) when an ITI of 30 s was used. A one-way repeated measures ANOVA with one within-subjects variable, treatment (MK-801 versus saline), was used to analyze performance during the 0.1 mg/kg MK-801 challenge when the ITI was 2 min.

Results

As indexed by days to criterion scores, training produced a wide range of acquisition stages in the rats (12–

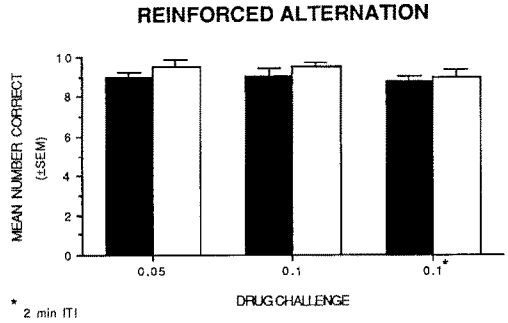

Fig. 1. Performance [mean ($\pm$SEM) number of correct responses] on the reinforced alternation task of rats that received injections of saline or MK-801 (0.05, and 0.1 mg/kg). Neither dose significantly affected performance relative to saline control levels, nor did lengthening the intertrial interval (ITI) from 30 s to 2 min following a 0.1 mg/kg challenge of MK-801. ■ MK-801 (mg/kg); □ Saline

28 days) and two rats did not reach criterion during the 30 days. The results of the MK-801 challenges (0.05 and 0.1 mg/kg) are depicted in Fig. 1. The two-way ANOVA on these data revealed no significant effects and there appeared to be no relationship between performance during drug testing and days to criterion scores achieved during training. In fact, the two rats that did not reach criterion during training, did so during drug testing. In addition, there was no effect of the 0.1 mg/kg challenge when the ITI was lengthened to 2 min. Rats were too impaired motorically to be tested following the 0.5 mg/kg dose of MK-801.

Experiment 2: Effects of MK-801 on working memory in the radial arm maze

Apparatus

The apparatus was an elevated (83.2 cm above the floor) eight-arm radial maze which has been described elsewhere (Wozniak et al. 1989). Briefly, it contained arms that were 70.2 cm long and 10.2 cm wide and which were connected to an octagonal central platform 48 cm in diameter. A Plexiglas barrier that was 29.2 cm high surrounded the central platform and contained eight doorways blocked by guillotine doors which could be manipulated simultaneously or individually by the experimenter.

Training

Ten, 180-day-old male rats were trained on a working memory protocol involving a win-shift spatial discrimination in an eight-arm radial maze (Hepler et al. 1985; Wozniak et al. 1989). A week before training began, a few chocolate chips were placed in the rats' cages on a daily basis in order to familiarize them with the reinforcer. Before training commenced, 5 days were devoted to shaping the rats to run down the alleys to eat a chip placed in the food cup at the end of each arm. Each training trial began by placing a rat on the central platform, raising the doors and allowing the rat to choose an arm. A choice was made when a rat placed its forepaws 17 cm beyond the doorway. After making a response, all doors were lowered except the door to the chosen arm. When the rat returned to the central platform, this door was lowered and the rat remained in the central area for 5 s, after which all the doors were raised and the rat was allowed to choose another arm. When the rat entered an arm and consumed a chocolate chip, a correct response was recorded. If the rat re-entered an arm where it previously consumed a chip during that trial, an incorrect response was recorded. The trial continued until the rat had consumed a chip in each arm or until 20 min elapsed from the beginning of the trial. Acquisition was defined by an a priori criterion of at least eight correct responses in the first nine responses for 4 consecutive days. Rats were subjected to drug testing after reaching the acquisition criterion in order to control for the possibility that the absence of a drug effect in the reinforced alternation experiment may have been due to "overlearning" (training beyond a reasonable acquisition criterion) in several animals.

Drug testing

Drug challenge procedures were initiated during the week after a rat had reached criterion and were continued in subsequent weeks according to the following weekly injection schedule. On Mondays, rats were tested but no injections were given. Rats were tested on Tuesdays and Fridays following challenges with MK-801 and Wednesdays and Thursdays following saline injections. The first two weekly protocols involved injecting 0.1 or 0.2 mg/kg MK-801, respectively, 30 min before testing animals on the win-shift (working memory) protocol described above. The next weekly challenge protocol involved 60 min pretrial injections of 0.1 mg/kg MK-801. The protocols for the following 2 weeks involved increasing the "memory load" of the task by interposing either a 90 s or a 15 min delay between the first four correct arm choices and subsequent choices. Thirty minute pretrial injections of 0.1 mg/kg MK-801 were used during these tests. The 15-min delay procedure involved testing in the absence of any injections on Monday and following an MK-801 challenge on Tuesday.

Statistical analyses

The efficiency (8/number of arm entries before responses were reinforced in all eight arms) of working memory performance following the 30-min pretrial injections of 0.1 mg/kg MK-801, were analyzed by a two-way ANOVA containing two within-subjects variables: treatment (MK-801 versus saline); and challenge (challenge 1 versus challenge 2). Several rats were unable to complete the test trial during the first 0.2 mg/kg MK-801 challenge. Due to the unequal *Ns* across the two 0.2 mg/kg challenges, working memory efficiency during each challenge was analyzed by an ANOVA involving one within-subjects variable: treatment (MK-801 versus saline). Response rates (arm entries per minute for the duration of the trial) for each of the above conditions (30-min pretrial injection, 0.1 and 0.2 mg/kg doses) were subjected to ANOVAs that were identical in design to the ones just described for the working memory data. The ANOVA used to analyze working memory efficiency from the 0.1 mg/kg challenge involving the 60 min pretrial injections was the same design as the one used for the 30 min pretrial injections at the same dose. In general, the delayed win-shift protocols were analyzed in a similar manner, except that the variable of pre versus postdelay efficiency (4/number arm entries before responses were reinforced in four arms) replaced the challenge variable described above.

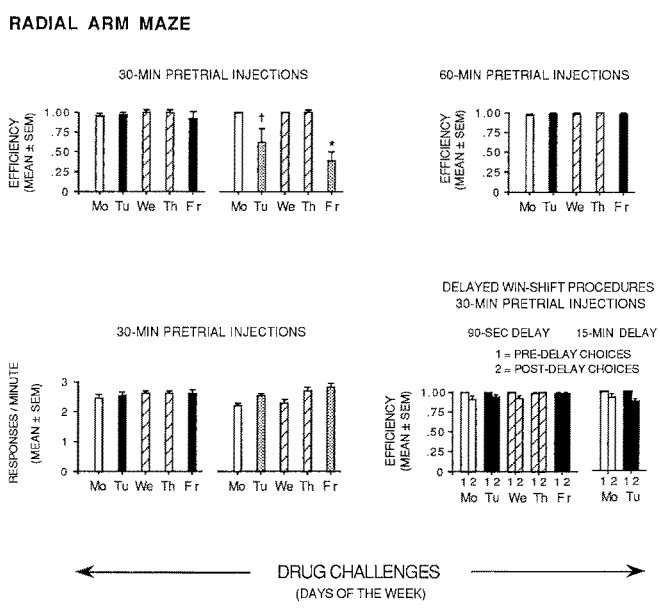

Fig. 2. Performance on the win-shift spatial discrimination in the radial arm maze as measured by mean (±SEM) efficiency [8/number of arm entries before eight correct (reinforced) responses were made]. Efficiency was impaired following the second challenge of 0.2 mg/kg MK-801 but sensorimotor disturbances (e.g., falling off the maze) were observed at this dose. Challenges of 0.1 mg/kg MK-801 did not impair efficiency regardless of whether 30-min or 60-min pretrial injections were used or whether a 90-s or a 15-min delay period was interposed between the first four correct responses and subsequent responses [efficiency = 4/number of arm entries before four correct (reinforced) responses were made]. □ No injection; ▨ Saline; ■ MK-801 (0.1 mg/kg); ▩ MK-801 (0.2 mg/kg); * $P<0.05$, $n=8/10$; † $n=5/10$

Results

No significant effects were found as a result of the analyses involving the 30-min and 60-min pretrial injections of 0.1 mg/kg MK-801 (Fig. 2, upper left and right corners). The 0.2 mg/kg dose (Fig. 2, middle, upper panel) produced apparent sensorimotor disturbances in the rats and during the first challenge, five of ten rats were unable to complete the test, since they fell off the maze. Evaluating the performance of these few animals did not reveal a significant drug effect. However, eight of ten rats remained on the maze and were able to complete testing following the second challenge at 0.2 mg/kg and the drug was found to produce a significant decrement in response efficiency in these animals [$F(1,7)=44.79$, $P<0.0005$]. No significant effects were found concerning response rates for the 30 min pretrial injections of 0.1 or 0.2 mg/kg doses of MK-801 (Fig. 2, lower left). An ANOVA on the working memory efficiency scores from the 90-s delay win-shift test (Fig. 2, lower right) revealed a significant effect of the delay (pre versus postdelay performance) on the first 0.1 mg/kg MK-801 challenge [$F(1,9)=7.23$, $P<0.025$], although the effect of drug and the drug by delay interaction were not significant. There were no significant effects as a result of the second challenge at the 90-s delay interval. Similarly, the results of the ANOVA on working memory efficiency from the 15 min delay win-shift procedure revealed a significant effect of the delay [$F(1,7)=2.33$, $P<0.02$], although the drug effect and drug by delay interaction were not significant (Fig. 2, lower right). In summary, there was no effect of MK-801 administration on working memory performance except at a dose which produced observable sensorimotor disturbances.

Experiment 3: Effects of MK-801 on sensorimotor function

Subjects

The ten rats used in experiment 3 (now 240 days old) served as subjects. Following termination of the radial maze tests of working memory, the rats were returned to continuous access to food and were not exposed to any drug or behavioral testing procedures for 30 days. Following this, the rats were subjected to a battery of tests to evaluate the effect of MK-801 on sensorimotor function or other capacities that may affect performance on learning and memory tasks. The battery was composed of tests which had been used previously to evaluate sensorimotor disturbances following systemic PCP administration (Kesner et al. 1981) or brain lesions (Finger et al. 1978; Whishaw et al. 1985; Wozniak et al. 1989).

Sensorimotor test battery

Plank. A rat was timed (s) for how long it could remain on a wooden plank that was 3 cm wide. Means for individual animals were calculated over two trials, each with a maximum time limit of 60 s.

Walking initiation. Pieces of tape were applied to the surface of a table to form a 50×50 cm square. A rat was placed in the middle of the square and was timed (s) for how long it took the rat to place all four paws outside the square.

Platform. A rat was timed (s) for how long it remained on an elevated (61 cm above the ground) platform (7.6×15.2 cm). A mean time was calculated over two trials for each animal with a maximum of 120 s for each trial.

Inclined screen. A rat was placed on a wire mesh grid (8 squares per 10 cm) which was inclined to 60°. Each animal was placed in the middle of the screen with its head oriented downward toward the floor and was timed (s) for how long it remained on the screen. A maximum time limit of 120 s was used.

Vibrissae touch. A cotton-tipped swab was used to brush the rats' vibrissae while they remained in their home

cages. Brushing was accomplished by bringing the swab from behind the animal to conceal it from view. If a rat oriented to the probe on at least two of three trials on each side, it received a plus.

Placing. A rat was lowered 3 times by its tail toward the edge of a black table. Whether sight of the table edge, touch of the vibrissae, or touch of the snout elicited forelimb extension was recorded.

Roll over-righting reflex. A rat was placed on its back and the presence or absence of a righting response was scored following release by the experimenter.

Free fall-righting reflex. A rat was held upside down 40 cm above a foam pad and then dropped. A score (+ or −) was given depending on whether the animal landed on its feet or not.

Drug testing

The rats were tested on the entire battery 5 times, one test being conducted every 6 days. The first test session was used to habituate the animals to the handling and equipment involved in the tests. Although these data are presented in the accompanying figures, they were not included in the statistical analyses. In the subsequent four tests, rats were injected IP with saline, 0.05, 0.1, or 0.2 mg/kg doses of MK-801, respectively, 25 min prior to testing.

Statistical analyses

The timed data from the sensorimotor battery were analyzed by a multivariate repeated measures analysis (Wilkinson 1987/SYSTAT) that included one within-subjects variable, dose (saline, and 0.05, 0.1, 0.2 mg/kg MK-801). The multiple dependent variables were the various tests (plank, walking initiation, inclined screen, and platform). Effects were further analyzed by appropriate pairwise comparisons. In order to address issues of test sensitivity and redundancy, one-way ANOVAs containing one within-subjects variable (dose) were planned for each test and subsequent pairwise comparisons were conducted following significant overall F ratios. Since the assumptions underlying statistical models involving repeated measurements (sphericity/compound symmetry) are frequently violated, both univariate and multivariate F ratios were computed for all effects involving within-subjects variables with more than two levels (Wilkinson 1987). The remaining tests (vibrissae touch, placing, roll over-righting reflex, free fall-righting reflex) involved dichotomous variables and were analyzed by either chi-square or Fisher's exact test.

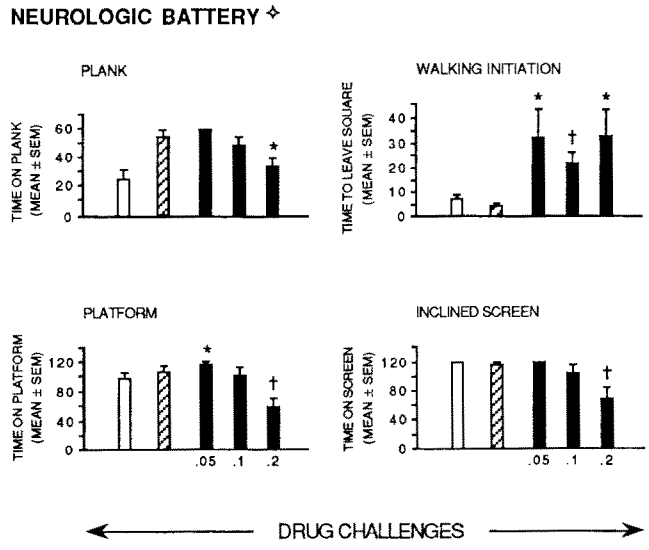

Fig. 3. Performance on certain tests from the sensorimotor battery. Significant pairwise comparisons involving performance following saline injections versus that following 0.05, 0.1, and 0.2 mg/kg doses of MK-801 are indicated for each test. The 0.2 mg/kg dose of MK-801 significantly affected performance on all the tests and each dose level significantly affected performance on the walking initiation test. The 0.05 mg/kg dose of MK-801 significantly affected performance on the platform test but this appeared to reflect an effect of practice in the absence of a drug effect. See Table 1 for other specific results of the statistical analyses. □ Habituation (saline); ▨ Saline; ■ MK-801 (mg/kg); ✧ all times measured in seconds; * $P<0.05$; † $P<0.01$

Results

Performance scores on each test for each dose level are presented in Fig. 3. The multivariate repeated measures analysis revealed a significant effect of dose [Hotelling-Lawley trace = 4.01, $F(3,7)=9.36$, $P<0.005$] and the dose by test interaction approached but did not reach significance [Hotelling-Lawley trace = 860.70, $F(9,1)=95.63$, $P<0.07$]. Univariate analyses indicated that 0.2 mg/kg MK-801 produced a significant effect on the tests relative to the saline challenge [$F(1,9)=5.95$, $P<0.037$] as did the 0.05 mg/kg dose [$F(1,9)=12.17$, $P<0.0007$]. The 0.2 mg/kg dose significantly impaired performance while performance on some of the tests may have still been improving in the absence of a drug effect at the 0.05 mg/kg dose.

The results of repeated measures analyses (univariate and multivariate) and pairwise comparisons for the individual tests from the battery are presented in Table 1. Both the univariate and multivariate overall F ratios were significant for the platform and inclined screen tests. The 0.2 mg/kg dose of MK-801 significantly decreased the time spent on the inclined plane (Fig. 3, lower right) relative to the saline challenge and the 0.1 mg/kg dose approached but did not achieve significance ($P<0.06$). Time spent on the platform was significantly reduced by the 0.2 mg/kg dose of MK-801 relative to the saline control performance. In contrast, there was

Table 1. The results of repeated measures analyses (univariate and multivariate) of rats' performances on certain tests from the sensorimotor battery following challenges with saline or MK-801 (0.05, 0.1, and 0.2 mg/kg). The results of pairwise comparisons involving performance following saline injections versus performances following each dose of MK-801 are also listed. See text for details of the individual tests and statistical analyses

Test	Univariate	Pairwise comparisons	Multivariate	
	*F*3,27 df *(P)*	*F*1,9 df *(P)*	H-L[a] Trace	*F*3,7 *df (P)*
Platform	9.91(0.0005)		2.25	5.26(0.03)
saline vs 0.05[b]		5.45(0.044)[c]		
saline vs 0.1		0.17(0.69)		
saline vs 0.2		12.05(0.007)		
Inclined screen	6.61(0.002)		2.22	5.18(0.03)
saline vs 0.05		1.00(0.34)		
saline vs 0.1		4.63(0.06)		
saline vs 0.2		12.39(0.007)		
Walking initiation	3.70(0.024)		1.45	3.37(0.08)
saline vs 0.05		5.68(0.041)		
saline vs 0.1		12.42(0.006)		
saline vs 0.2		7.38(0.024)		
Plank	4.12(0.016)		0.92	2.15(0.18)
saline vs 0.05		2.15(0.18)		
saline vs 0.1		0.42(0.54)		
saline vs 0.2		6.67(0.03)		

[a] Hotelling-Lawley Trace statistic
[b] Doses of MK-801
[c] Performance under drug significantly better than under saline. See text for details

a significant increase in the time spent on the platform (Fig. 3, lower left) during 0.05 mg/kg MK-801 challenge relative to the saline performance reflecting a continued practice effect in the absence of a drug effect. The overall univariate *F* ratio was significant for the walking initiation test (Fig. 3, upper right) while the multivariate F approached but did not achieve significance ($P<0.08$). The overall univariate *F* was significant for the plank test (Fig. 3, upper left) but the multivariate *F* was not. Pairwise comparisons indicated that all of the doses of MK-801 resulted in significant increases in the time to leave the square in the walking initiation test while only the 0.2 mg/kg dose produced a significant performance decrement on the plank test.

No significant treatment effects were found for the tests involving dichotomous variables (vibrissae touch, placing, roll over-righting reflex, free fall-righting reflex).

In summary, 0.2 mg/kg MK-801 was found to impair performance on several of the tests within the sensorimotor battery. Although the effect of the 0.05 mg/kg dose of MK-801 was also significant, at least part of this effect resulted from a continued improvement in performance due to practice in the absence of a drug effect (e.g., data from the platform test). In addition, the platform, inclined screen, and plank tests manifested similar dose-response functions which were different in nature from the dose-response relationship shown in the walking initiation test, possibly indicating drug effects on different functions.

Experiment 4: Effects of MK-801 on retention of a position habit reversal

Shaping

Thirty-eight 190-day-old rats were trained to acquire and reverse a position habit in the T-maze described in experiment 1. Before drug testing commenced, the rats were subjected to 4 days of shaping in order to habituate them to the maze and handling procedures and to ensure that they spent equal amounts of time consuming the reinforcer in both wings.

Drug testing

Rats were tested on a modified version of the 3-day position habit reversal protocol described by Handelmann et al. (1987). On day 1 of the 3-day test sessions, rats were reinforced for choosing only one side of the T-maze. Rats were run until they made nine out of ten consecutive correct (reinforced) responses. On day 2, half of the rats were injected IP with 0.1 mg/kg MK-801 and half with saline. Fifteen minutes later they were trained to choose the side of the T-maze opposite from the one in which they were reinforced on the previous day. Again, rats were required to make nine out of ten correct responses to the reinforced (reversed) side. On day 3, rats were given a one-trial test to see which side

they would choose, i.e., to see if they remembered what they had learned on day 2. A correct response was defined as choosing the side which they had been reversed to (reinforced in) on day 2. On day 1, individual rats were trained with massed trials in effect and the test sequence across individual animals was randomly determined. The side in which reinforcement occurred on day 1 was randomly determined across animals within a counterbalanced design (half of the rats were reinforced on the right and half on the left). On days 2 and 3, rats were tested in pairs (matched according to trials to criterion on the 1st day of the position habit training) and the order of testing was randomly determined for each trial and across pairs of animals. The wings were cleaned with a scented detergent after each trial on all days. Rats were run in two squads each week: one squad on Monday through Wednesday; and the other on Wednesday through Friday. Rats were randomly assigned to squads and were assigned to treatment groups by "matching" on the basis of trials to criterion on day 1 of the saline only challenge in phase 1 (described below).

The above protocol was repeated once a week for 5 consecutive weeks for each of two phases (phase 1 and phase 2) of the experiment. The first challenge in each phase involved saline injections for all rats, while subsequent challenges involved MK-801 (0.1 mg/kg) for one group and saline for the other. After the last challenge of phase 1, the rats were returned to continuous access to food for 19 days. Following this they were again placed on restricted access to food for 1 week before phase 2 commenced. Thus, the rats spent 26 days without being subjected to any drug or behavioral testing. The protocol for phase 2 was identical to the one used in phase 1, except that the groups were "crossed-over" with regard to the drug treatment. Thus, the rats that were previously challenged with saline were now challenged with MK-801, while the opposite was true for the rats that received MK-801 in phase 1.

Statistical analyses

In general, the data were analyzed in three ways. In one overall analysis, the data from the two phases of the experiment were combined as if the same experiment were replicated for each group. Two additional analyses were carried out, one on each group, to assess whether the order of treatment (MK-801 versus saline) had an effect on performance. Thus, "cross-over" effects were evaluated by using a treatment nested within groups design. Each type of design involved two within subjects variables: treatment (MK-801 versus saline); and challenges (1–4); and the overall analysis also contained one-between subjects variable (groups A and B). A repeated measures analysis of categorical data (CATMOD/SAS) was used to evaluate the correctness of response data on the one-trial test conducted on day 3, while trials to criterion and response latencies were analyzed by univariate and multivariate repeated measures analyses (Wilkinson 1987/SYSTAT).

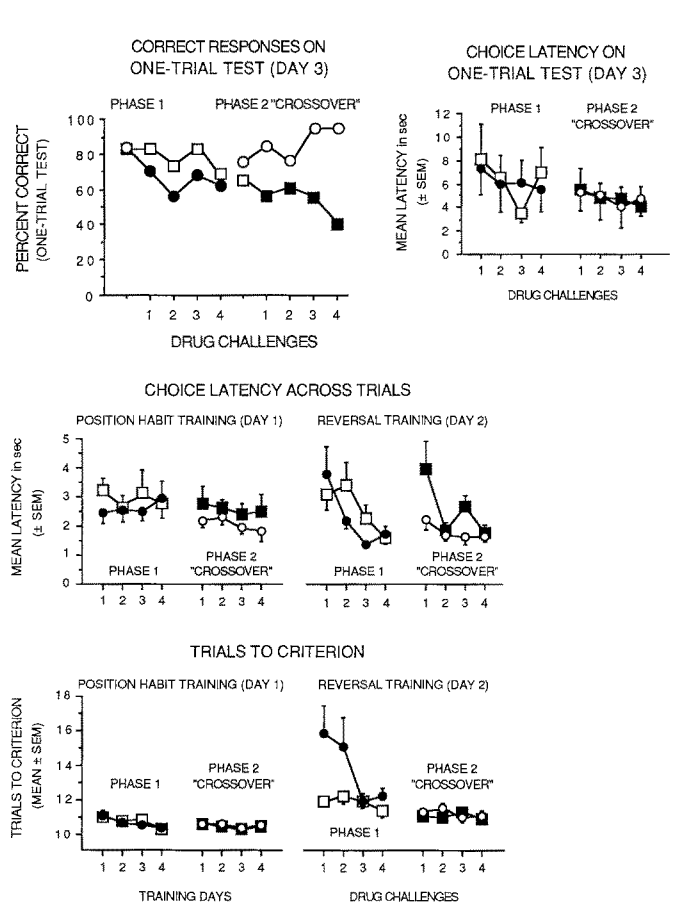

Fig. 4. Performance on several variables related to the position habit reversal task as a function of injections of saline versus challenges with 0.1 mg/kg MK-801. The panel on the upper left shows that each group of rats, when compared with itself across phases, made significantly fewer correct responses during the phase in which it received MK-801 than when it received saline. This within-subjects effect was significant regardless of whether MK-801 was received in the first phase or in the second. Neither group displayed significant drug effects with regard to choice latency on the one-trial test (*upper right*). Another important effect depicted in the figure involves the trials to criterion data (*bottom panel*). During reversal training, there appeared to be an interaction between drug effects and familiarity with the task, in that group A required more trials to criterion than group B in phase 1 but there were no differences between the groups in phase 2 (see text for further discussion). Group A: ● MK-801 (0.1 mg/kg); ○ Saline. Group B: ■ MK-801 (0.1 mg/kg); □ Saline

Results

The correctness of response data from the one-trial test (day 3) is depicted in Fig. 4, upper left. When the data were combined as if the same experiment were replicated for each group, the CATMOD/SAS analysis revealed a significant treatment effect (MK-801 versus saline) (Chi-square = 29.34, $df = 1$, $P < 0.00009$) and a nonsignificant treatment by challenge interaction. Thus, across challenges, significantly fewer MK-801-treated rats chose (recalled) the side they had been reversed to on

day 2 compared to saline controls. The analysis also revealed that performance significantly differed across challenges (Chi-square = 9.02, $df = 1$, $P < 0.029$), and that one group performed significantly different from the other (Chi-square = 3.86, $df = 1$ $P < 0.0495$). In the follow-up analyses of "cross-over" effects, contrasts constructed within this design (treatments nested within groups) revealed a significant treatment effect in each group [Chi-square = 13.40, $df = 1$, $P < 0.0003$ (group A); Chi-square = 8.98, $df = 1$, $P < 0.0027$ (group B)]. Thus, MK-801 significantly impaired performance on the one-trial test on day 3 regardless of whether rats were first challenged with saline and then MK-801 or vice versa.

In general, the two groups performed equivalently in terms of trials to criterion during position habit training (day 1) across both phases of the experiment (Fig. 4, lower left). The overall analysis containing both groups revealed that the effects of groups and treatment were not significant. However, the groups × treatment interaction was significant [$F(91,35) = 30.76$, $P < 0.0009$] and the univariate and multivariate F ratios for the effect of challenge were both significant [$F(3,105) = 3.27$, $P < 0.024$, Hotelling-Lawley Trace = 0.46, $F(3,33) = 5.01$, $P < 0.009$]. In the individual analyses involving each group, a significant effect of treatment [$F(1,18) = 14.41$, $P < 0.001$] and a significant effect (univariate only) of challenge were found for group A. Only the effect of treatment was significant [$F(1,17) = 16.23$, $P < 0.001$, for group B]. Since no injections were given during position habit training and since each group required fewer trials to criterion during phase 2, it seems likely that the effect of treatment in the individual analyses may reflect a practice effect across the two phases of the experiment. This is consistent with the significant groups × treatment interaction in the overall analysis including both groups.

From an inspection of the trials to criterion data for reversal training (day 2) depicted in Fig. 4, lower right, it appears that there was an interaction between familiarity with the task and drug (MK-801) effects as well as a practice effect across phases of the experiment. The overall analysis containing both groups indicated a significant effect of groups [$F(1,35) = 6.89$, $P < 0.013$], treatment [$F(1,35) = 4.66$, $P < 0.038$] and a significant group × treatment interaction [$F(1,35) = 17.32$, $P < 0.0009$], as well as a significant (univariate F only) effect of challenge [$F(3,105) = 2.77$, $P < 0.046$]. A significant treatment (phase 1 versus phase 2) effect was found in the individual analyses for each group [group A, $F(1,18) = 11.31$, $P < 0.003$; group B, $F(1,17) = 13.54$, $P < 0.002$]. Since the trials to criterion decreased for both groups during phase 2, it appears that at least a portion of the treatment effect was due to practice. However, additional contrasts comparing the group performances of MK-801-treated rats with saline controls during each phase of the experiment indicated that the drugged rats took significantly more trials to reach criterion than controls during reversal training in phase 1 [$F(1,36) = 6.75$, $P < 0.013$], but not in phase 2. Thus, portions of the significant group, treatment, and group × treatment interaction effects in the overall analysis may reflect effects of practice and order of drug treatment. Most importantly, MK-801 appeared to affect performance only when the animals had limited experience on the behavioral task.

Latency on the one-trial test (day 3) was unaffected by any variable in the experiment (Fig. 4, upper right). Mean latencies during position habit training (day 1) were generally unaffected by most of the variables (Fig. 4, middle left). For example, the group × treatment interaction was the only significant effect in the overall analysis containing both groups [$F(1,35) = 8.18$, $P < 0.007$]. Mean latencies were significantly greater in phase 1 than in phase 2 for group A [$F(1,18) = 8.34$, $P < 0.010$], but this was the only significant effect in the individual analyses involving each group. In general, the mean latencies during reversal training (day 2) seemed to decrease across challenges within phases, especially for the MK-801-treated rats (Fig. 4, middle right). In the overall analysis, there was a significant effect of challenge [$F(3,105) = 8.18$, $P < 0.0009$, Hotelling-Lawley Trace = 0.618, $F(3,33) = 6.79$, $P < 0.009$], and a significant (univariate F only) treatment × challenge interaction [$F(3,105) = 3.00$, $P < 0.034$]. In the individual analyses involving each group, there was a significant effect of treatment [$F(1,18) = 5.40$, $P < 0.032$] and challenge [$F(3,54) = 4.96$, $P < 0.004$, Hotelling-Lawley Trace = 1.149, $F(3,16) = 6.13$, $P < 0.009$], as well as a significant (univariate F only) treatment × challenge interaction [$F(3,54) = 3.10$, $P < 0.034$] for group A. For group B, only the effect of challenge was significant [$F(3.51) = 4.11$, $P < 0.011$, Hotelling-Lawley Trace = 0.847, $F(3,15) = 4.237$, $P < 0.02$]. Thus, mean latencies decreased across challenges within each phase of the experiment, especially for MK-801-treated rats.

In summary, on the one-trial test, significantly fewer MK-801-treated rats chose (recalled) the side of the T-maze they had been reversed to while under the influence of the drug on the previous day. Significant "cross-over" effects involving trials to criterion and response latencies during reversal training and latencies during position habit training suggest interactive effects between MK-801 administration and familiarity with the behavioral protocols.

Discussion

The results of the present study suggest that, at a nonintoxicating dose (0.1 mg/kg), MK-801 may have relatively selective effects on learning/memory since the drug affected performance on one type of a task but not on another type. At this dose, MK-801 did not impair performance on two tasks (reinforced alternation and radial arm maze) used to assess working (trial-dependent) memory. In these two tasks, rats were challenged with MK-801 after they had been trained for many days and demonstrated learning by meeting an acquisition criterion which required consistently accurate performance over a 4 day period. Whether rats could recall, at a later point in time, new information learned while under the

influence of MK-801, was not evaluated in these protocols. However, this aspect of memory was examined in the position habit reversal task. In this task, rats were required, while under the influence of MK-801 (0.1 mg/kg), to execute a response that was spatially opposite from the one learned on the previous day. Although the rats demonstrated learning (reached criterion) during this reversal training, when tested on the following day, they performed as if they did not recall what they had learned (i.e., the reversal) while under the influence of the drug. Although performance on one sensorimotor test (walking initiation) was significantly altered by MK-801 at 0.1 mg/kg, this does not confound interpretation of the position habit reversal data, since animals showed evidence of learning while under the influence of this dose of MK-801 during reversal training and only evidenced impairment when challenged to recall the learned information 1 day later (after the drug effects had worn off). It should be noted that the data from the position habit reversal task could possibly be explained in terms of state dependent learning; our experimental design does not rule out this possibility.

Although MK-801 did not affect performance on the working memory protocol in the radial arm maze at 0.1 mg/kg, performance was significantly impaired following the second 0.2 mg/kg challenge. During the first challenge at 0.2 mg/kg, only five of ten rats were able to remain on the maze and complete the test and the limited power resulting from the small number of subjects was probably responsible for the absence of a drug effect following this challenge. In contrast, eight of ten rats completed the test during the second 0.2 mg/kg challenge and these rats demonstrated a significant performance decrement. These results are similar to those of Mondadori et al. (1988), who reported that performance on the working memory protocol in the radial maze was significantly impaired when gerbils were challenged with 0.3 mg/kg MK-801 but not when 0.1 mg/kg was used. Our impression from the radial arm maze study that MK-801 at 0.2 mg/kg caused significant sensorimotor impairment was confirmed by the results of the sensorimotor test battery in which this dose of MK-801 significantly impaired performance on several of the tests (plank, inclined screen, platform, and walking initiation). Thus, it is unclear whether the impaired performance in the radial arm maze exhibited by rats treated with 0.2 mg/kg MK-801 reflects an effect of the drug on memory or an acute intoxicational effect on sensorimotor systems. Similar caveats may be appropriate for interpreting impaired performance on the working memory protocol in the radial maze following relatively high doses (6 mg/kg) of PCP (Kesner et al. 1983; McCann and Winter 1986), since Danysz et al. (1988), employing a similar test, found that a lower dose of PCP (4 mg/kg) induced stereotypy which disrupted performance.

In view of the psychotomimetic effects of PCP and related compounds, it must be assumed that these agents have the ability to interfere with complex sensory information processing. Consistent with this assumption, Salt and Eaton (1988) reported that somatosensory messages triggered by hair follicle stimulation or nociceptive stimuli are disrupted by microelectrophoretic application of either competitive or noncompetitive NMDA antagonists onto the surfaces of thalamic neurons that ordinarily receive and relay these messages to the cortex. Moreover, we recently found (Olney et al. 1989) that MK-801, at a relatively low dose ($ED_{50} = 0.18$ mg/kg for females; 0.32 mg/kg for males), induces frank pathomorphological changes in specific populations of cerebrocortical neurons whose functions include sensory information processing and emotional reactivity. There does not appear to be a very wide margin between doses of MK-801 that affect memory and doses that may alter other information processing modalities. Such findings suggest the need for caution in interpreting experiments in which intoxicating doses of these agents are used to study effects on memory and learning. According to our observations, any dose of MK-801 in the range of ≥ 0.2 mg/kg should probably be considered grossly intoxicating.

Morris (1988) has suggested that some of the sensorimotor disturbances observed during water maze testing of rats treated ICV with AP5 might be attributed to a combination of drug effects and lack of familiarity with the behavioral task. Similarly, we observed during the position habit reversal experiment, that a dose of MK-801 (0.1 mg/kg) that did not cause gross intoxication was associated with certain performance effects, apparently reflecting an interaction between drug treatment and degree of familiarity with the task. In this experiment, conducted by cross-over design, the treatment sequence for one group of rats was MK-801 in the first phase and saline in the second, whereas the sequence for the other group was saline first and MK-801 second. The group that received MK-801 in the first phase had greater response latencies during both position habit and reversal training, and manifested perseverative tendencies and required more trials to criterion during reversal training, than was the case for these animals in phase 2 when they received saline (but were also more familiar with the task). Thus, either a drug effect in phase 1 or greater familiarity with the task in phase 2 might be postulated to explain the performance differences across phases. However, the other group of rats did not show these across-phase effects, despite being unfamiliar with the task in phase 1 and receiving MK-801 in phase 2. These findings suggest an interaction between drug effects and task familiarity, since neither variable acting independently can account for these results.

It is important to note that although the performance of one group was affected by the acute influence of MK-801 on day 2, both groups learned the habit reversal on that day, then seemed not to remember the learned information when challenged to recall it in the single-trial test on day 3. Since this apparent memory deficit was shown by both groups on day 3 if treated on day 2 with MK-801 (but not saline), and at the time of displaying this deficit, one group was familiar and the other unfamiliar with the task, the deficit appears to be a drug-related effect which is not dependent on familiarity with

the task. Thus, we propose that the performance deficit in the single-trial test on day 3 can be viewed as an effect of MK-801 on memory formation which is unrelated to the observed performance effects attributable to an interaction between acute drug effects and task familiarity. Furthermore, the present experimental design permits a dissociation of drug-related memory effects from other subtle drug-related effects that vary with degree of task familiarity and may or may not relate to a primary effect on memory.

The consensus of prior studies is that NMDA antagonists disrupt memory formation. However, many of these results should be interpreted cautiously since, in some cases, invasive methods (intracerebral infusion) were used for drug delivery and in the majority of studies, regardless of mode of drug administration, the doses of NMDA antagonist required to induce a performance deficit may have been high enough to disrupt various sensorimotor or cognitive functions, making it unclear to what extent the performance deficit could be attributed to an impairment in memory. In the present study, an intoxicating dose of MK-801 was required to disrupt performance on a spatial working memory task in the radial arm maze in rats that had demonstrated a consistent high-degree of proficiency in performing the task. However, we found that memory acquisition or consolidation of new information was disrupted by a nonintoxicating dose of MK-801. Our results are consistent with very recent findings pertaining to MK-801 (Robinson et al. 1989; Whishaw and Auer 1989) and with those pertaining to the noncompetitive NMDA antagonist, PCP (Handelmann et al. 1987) and the competitive antagonist, AP5 (Staubli et al. 1989).

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