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The Effects of MK-801 on Spatial Working Memory and Within-Session Spatial Learning

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WHITE, A. M. AND P. J. BEST. *The effects of MK-801 on spatial working memory and within-session spatial learning.* PHARMACOL BIOCHEM BEHAV **59**(3) 613–617, 1998.—The present study investigated the effects of the NMDA channel blocker MK-801 (0.05, 0.10, and 0.15 mg/kg) on a task that allows for the assessment of both spatial working memory and within-session spatial learning. During the first trial of each day, subjects were shown the spatial location of a food reward on a six-arm radial-arm maze. During nine subsequent free-choice trials, subjects were reinforced for returning to that same spatial location. The location of the food reward varied across days. Thus, choosing correctly on any given trial required subjects to remember where food had been received during the previous trials of that day. The effects of MK-801 on working memory were assessed by analyzing the overall number of errors committed during the nine free-choice trials of each day. The effects of MK-801 on within-session learning were assessed by comparing the number of errors committed during the first three trials of each day to the number of errors committed during the last three trials of each day. Only the highest dose of MK-801 tested (0.15 mg/kg) impaired spatial working memory. No dose of MK-801 impaired the ability of subjects to acquire spatial information within a given session. The failure of MK-801 to impair within-session spatial learning stands in contrast to the well-known effects of MK-801 on spatial learning measured across days. Thus, when coupled with previous research, the findings of the present study further suggest that the NMDA receptor plays a role in the long-term, but not short-term, storage of spatial information. © 1998 Elsevier Science Inc.

MK-801 Spatial Learning Memory NMDA

THE N-methyl-D-aspartate receptor (NMDA), a receptor subtype for the excitatory neurotransmitter glutamate, has received a great deal of attention in recent years. Activity at the NMDA receptor has been linked to neuronal death due to a variety of insults, including epileptiform activity, anoxia, ischemia, and hypoglycemia (15). In addition, considerable evidence suggests that the NMDA receptor plays a pivotal role in the induction of long-term potentiation (LTP), a rapidly induced and long lasting form of synaptic plasticity hypothetically linked to learning (3).

Although NMDA receptors are located throughout the brain in varying concentrations, the density of these receptors is greatest in the hippocampus (4,10), a brain region critically involved in spatial information processing (12). Converging evidence suggests that the NMDA receptor, and perhaps a form of synaptic plasticity akin to LTP, plays a crucial role in the acquisition of spatial information. NMDA antagonists, such as the noncompetitive channel blocker MK-801 [(+)-10,11-dihydro-5-methyl-5H-dibenzo[a,d] cycloheptene-5,10 imine], block the induction of hippocampal LTP in vivo (1) and in vitro (18). NMDA antagonists also impair the learning of spatial working and reference memory tasks when learning is assessed by measuring changes in performance across several days (9,16). However, NMDA receptor activation does not appear to be necessary for spatial learning when learning is measured within a single day (8).

In contrast to the role of the NMDA receptor in the induction of LTP and in spatial learning measured across days, NMDA receptor activation is not necessary for the expression of hippocampal LTP (18), nor is it thought to be necessary for the performance of previously learned spatial tasks (2,6). Although working and reference memory impairments have been reported to occur following the administration of NMDA antagonists, such impairments typically emerge at doses much higher than those needed to impair learning. For instance, while MK-801 impairs the acquisition of a spatial working memory task at a dose of 0.0625 mg/kg, a dose of 0.125 mg/kg is needed to impair performance when subjects are trained to criterion prior to drug administration (16,17).

The present study examines the effects of the NMDA channel blocker MK-801 on the performance of a task that allows for the concurrent assessment of spatial working memory and within-session spatial learning. Based upon the find-

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ings of previous research, it is hypothesized that 1) if MK-801 impairs spatial working memory on this task, doses greater than 0.1 mg/kg will be required to do so; and 2) NMDA receptor blockade should not disrupt the ability of subjects to acquire spatial information within a given day.

METHOD

Apparatus

A four-arm radial-arm maze, used during adaptation, was located in the center of a laboratory room rich with distal cues. The maze was 90 cm above the floor with four arms (50 cm long and 12 cm wide) radiating outward at 90° angles.

An elevated six-arm radial-arm maze, used during pretraining, training, and testing, was positioned in the center of a novel laboratory room rich with distal cues. The maze was 93 cm above the floor with six arms (48 cm long and 12 cm wide) radiating outward from a central platform at 60° angles. Plexiglas walls on each maze arm, roughly 7.5 cm in height, slanted outward forming V-shaped troughs. Metal food dishes were located at the end of five of the six maze arms. Removable wooden barriers, placed at the junction between the maze arms and the central platform, were used to block entry to selected arms.

Subjects

Eight male Long–Evans hooded rats from Charles River were housed individually in hanging steel cages in an approved animal colony and maintained on a 12 L:12 D cycle (lights on at 0700 h). Handling and training occurred between 0900 and 1800 h. Two weeks prior to pretraining, access to food was restricted to 1 h per day. Animals were handled and weighed daily to ensure that body weight did not drop below 80% of their free-feeding weight. At the time of pretraining, all subjects weighed between 250–375 g. Animals were never water deprived.

ADAPTATION

Adaptation took place on the four-arm radial-arm maze. Pieces of Froot Loops cereal were scattered throughout the maze and were located in metal food dishes at the end of each maze arm. On 3 consecutive days, animals were given three 120-s trials followed by feeding.

Pretraining

Pretraining took place on the six-arm radial-arm maze. Ten trials were conducted on each of 12 days. During each trial, barriers were placed at the entrance to all arms except for the start arm and goal arm. The goal arm was defined by its spatial location (i.e., position relative to distal cues). The same goal arm was used for all 10 trials of a given day. The goal arm changed across days. Thus, the same spatial location was rewarded for all trials of a given day, but the rewarded spatial location changed across days. Start arms varied on each trial such that each arm, with the exception of the goal arm, served as the start arm exactly twice per day. The order of start arms used within each day, as well as the order of goal arms used across days, was assigned using a digram-balanced Latin square design (7).

At the beginning of each trial, two small broken pieces of Froot Loops cereal were placed in the metal food dish at the end of the goal arm. Barriers were placed at the entrances to all but the start and goal arms. Subjects were placed at the distal end of the start arm, facing away from the center of the maze. A trial ended when a subject obtained the food reward at the end of the goal arm or when 90 s expired, whichever came first. Latencies to reach the food cup were monitored using a stopwatch and were recorded at the end of each trial. Trials were separated by an approximately 10–20-s interval, during which time subjects were placed in a holding cage and the maze was prepared for the next trial.

Training

The training procedure was similar to the pretraining procedure with the exception that, following the initial forced trial of each day, subjects received nine trials with the barriers removed, allowing access to all maze arms (free-choice trials). During free-choice trials, the animal was rewarded for returning to the location in which food was received during the forced trial (the goal arm). Proceeding to a food cup located at the end of any other arm constituted an error. In the event of an error, the subject was removed from the maze, wooden barriers were placed at the entrance to all but the start arm and goal arm, and the trial was rerun. To ensure that animals did not learn to locate the goal arm based upon scent trails or other intramaze cues, the maze was rotated twice per day (prior to the fourth and eighth trials).

Subjects were trained to a criterion of 2 consecutive days of at least seven out of nine correct responses during free-choice trials. The day following achievement of criterion, subjects were administered a 2.0 ml/kg IP injection of saline 30 min prior to training. The volume of the injection corresponded to the volume of the medium dose of MK-801 (0.1 mg/kg). If the subject continued to perform at a level of seven out of nine correct choices or above, he advanced to the testing phase. If performance fell below seven out of nine correct choices, the subject continued to receive saline injections on subsequent days until performance returned to seven out of nine correct responses or above. At such time, a day off was given and the subject advanced to the testing phase.

Testing

A within-subjects design was utilized to test each subject under four doses of MK-801 (saline control, 0.05, 0.1, and 0.15 mg/kg). Subjects were randomly assigned, without replacement, to eight treatment sequences determined by a digram-balanced Latin square design (7).

Subjects were retrained under saline until performing at a level of seven out of nine correct responses or above during free-choice trials. The following day, subjects were tested under the corresponding dose of MK-801. The testing procedure was similar to the training procedure with the exception that the appropriate dose of MK-801 was administered 30 min prior to the day's session. Following a day off, subjects were once again retrained under saline to a level of seven out of nine correct responses or above and then retested under a different dose of MK-801. This cycle was repeated until subjects received all four doses of MK-801. During testing, as during pretraining and training, subjects were allowed a maximum of 90 s to complete each trial. In all conditions, subjects responded within the allotted 90 s period.

Food on Demand (FOD) Probe Test

Following the completion of testing under all four doses of MK-801, subjects were retrained to criterion and administered an FOD probe test (16). The FOD procedure was similar to the procedure used during normal testing with the exception that food was not placed in the food cup at the end of

the goal arm until a subject progressed to the end of that arm. If, during training, subjects had learned to choose the correct arm by detecting the presence of food in the food cup, subjects should commit more errors during the FOD probe test than during normal testing under saline.

Operational Definitions and Statistical Analyses

According to Olton (13), working memory is required in "any situation which involves the repetition of a general set of rules and procedures, but variation in the items to which these are applied." In the present task, the general set of rules and procedures included the following: 1) on every trial, there was food available at the end of one of the maze arms; and 2) food was available in the same location during all trials of a given day. The item of information that varied (and, therefore, had to be stored in working memory) was the location of the food reward during each particular day. Accordingly, entries into arms other than the goal arm during free-choice trials were considered errors in working memory. The effect of drug dose on the number of working memory errors committed during testing was assessed using a one-way within-subjects ANOVA. A significant main effect revealed by the ANOVA was followed by paired *t*-tests.

In addition to allowing for the assessment of spatial working memory, the task used in the present study also allows for the assessment of within-session learning. Because subjects are rewarded in the same location for 10 consecutive trials, information regarding the location of the food reward should accumulate across trials. If such is the case, the frequency of errors should decline as the session progresses. To assess the effects of MK-801 on within-session learning, errors committed during testing were separated into those committed during the first three (block 1), second three (block 2), and last three (block 3) free-choice trials. A two-way (dose × trial block) within-subject ANOVA was performed on the data from block 1 and block 3. Within-session learning was thus defined as a significant decrease in the number of errors committed between the first three (block 1) and last three (block 3) free-choice trials. Significant main effects revealed by ANOVA were followed by paired *t*-tests. In addition, a one-way within-subjects ANOVA was used to compare the number of errors committed under the four doses of drug during the third block of trials.

A digram-balanced Latin square design was used in the present study to control for the possibility of order effects (7). Drug doses were administered in a different order for each animal, and each dose preceded and followed each other dose exactly once. By collapsing over doses and analyzing performance according to day-of-treatment, this design permits assessment of a drug-practice effect (i.e., tolerance or experience). To determine if the repeated exposure to MK-801 influenced performance, scores from the four days-of-treatment were analyzed using a one-way within-subject ANOVA.

A one-way within-subjects ANOVA was used to assess the effect of drug dose on running latencies. A significant main effect was followed by one-tailed *t*-tests for repeated measures. A *t*-test for repeated measures was also used to compare performance during normal testing under saline to performance during the FOD probe test.

RESULTS

MK-801 produced a dose-dependent effect on the number of working memory errors committed during testing [one-way repeated measures ANOVA, $F(3, 21) = 3.13$, $p < 0.05$; see Fig. 1]. The significant main effect was due to a larger number of errors committed under the high dose of MK-801 than under either saline, $t(7) = 2.14$, $p < 0.05$, or medium MK-801, $t(7) = 2.20$, $p < 0.05$).

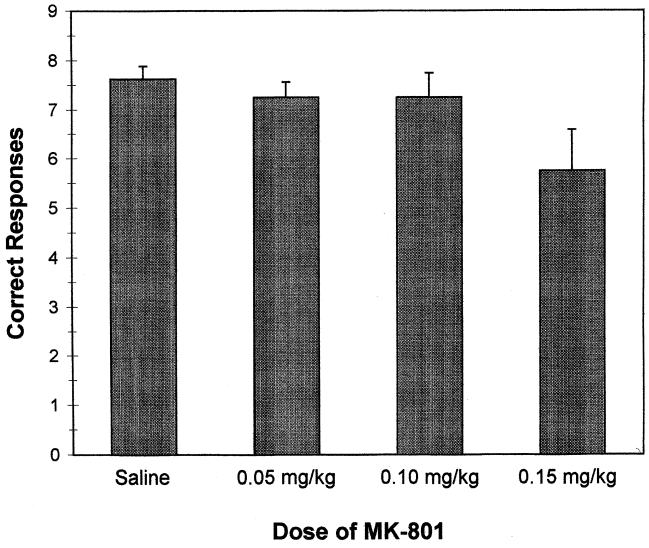

FIG. 1. Performance on the working memory task under four doses of MK-801. Only performance under the high dose differed significantly from performance under saline.

MK-801 did not disrupt within-session learning. Specifically, regardless of drug dose, subjects committed fewer errors during the last three trials of testing (block 3) than during the first three trials of testing (block 1) [two-way (dose × trial-block) repeated measures ANOVA; effect of trial-block, $F(1, 14) = 35.85$, $p < 0.0001$; no significant dose × block interaction, $F(6, 42) = 0.59$, $p > 0.05$; see Fig. 2]. One-tailed *t*-tests indicated that subjects in all conditions did, in fact, commit fewer errors during the last block of trials than during the first block (all *p*-values less than 0.05).

The number of errors committed under the four doses of MK-801 did not differ during the final block of trials, $F(3, 21) =$

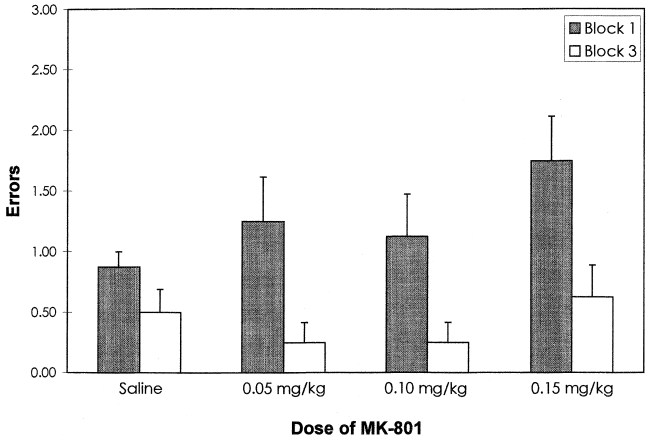

FIG. 2. The average number of errors committed during the first three and last three trials of testing. Under all four doses of MK-801, subjects committed significantly more errors during the first three than the last three trials (all *p*-values less than 0.05).

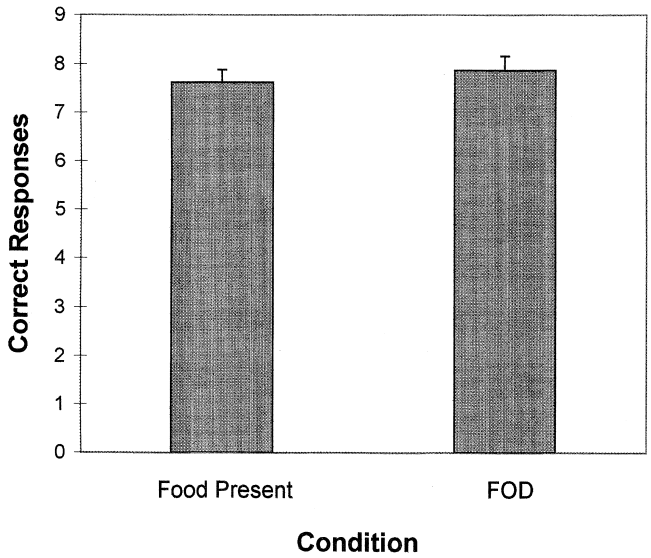

FIG. 3. Comparison between the performance of subjects during normal testing under saline and during the FOD probe test.

1.09, $p > 0.05$. Thus, by the end of the session, subjects in all conditions were performing at a comparable level.

To determine if the repeated exposure to MK-801 influenced performance, scores from the four days-of-treatment were analyzed using a one-way repeated measures ANOVA. Overall performance did not change across days-of-treatment, $F(3, 21) = 2.20, p > 0.10$. Thus, if tolerance did develop due to repeated administration of MK-801, it did not influence performance on the task.

Running latencies did not differ during testing under the four doses of MK-801 [repeated measures ANOVA; $F(3, 21) = 2.83, p > 0.05$].

Performance of subjects during the FOD probe test did not differ from performance during normal testing under saline, $t(7) = -0.067, p > 0.05$; see Fig. 3]. These results strongly suggest that subjects were not using the odor of the food reward to locate the goal arm.

DISCUSSION

Only the highest dose of MK-801 (0.15 mg/kg) impaired spatial working memory in the present task. These findings are consistent with previous research examining the effects of MK-801 on spatial working memory (16,19,20). The reason for the impairment produced by the high dose of MK-801 is unclear. Although the impairment may reflect a role for the NMDA receptor in the processes underlying working memory per se, the impairment may also be due to nonmnemonic behavioral effects of the drug. All subjects tested under the high dose, and a few tested under the medium dose, exhibited obvious behavioral changes. Although no formal tests were conducted, reactions to the high dose appeared to include some degree of ataxia and hyperactivity. In addition, a few subjects exhibited pronounced hyperreactivity (e.g., being startled by attempts to remove the rat from the maze or out of the holding cage) and engaged in stereotypic behaviors during testing (e.g., circling in the distal end of the start arm or repeatedly exiting and reentering the start arm). Similar observations have been reported following doses of MK-801 much lower than the high dose used here (5).

No dose of MK-801 impaired the within-session learning of spatial information. Specifically, under all doses of MK-801, subjects committed more errors during the first block of trials than the last block of trials. In fact, the number of errors committed under the four doses of MK-801 during the last block of trials did not differ. Thus, even though the high dose of MK-801 produced an overall impairment in performance, it did not prevent subjects from processing and storing spatial information. Such results are interesting in light of evidence that even the lowest dose of MK-801 used here (0.05 mg/kg) impairs spatial learning when spatial learning is measured across days (14,19).

Similar accounts of spared within-session learning have been reported in the literature. Kesner and Dakis (8) observed that intrahippocamal administration of MK-801 impaired the learning of a spatial task across days but did not disrupt the within-session acquisition of spatial information. Similarly, Morris (11) reported that, although AP5 impaired learning in the water maze across days, subjects exhibited significant within-session acquisition. Further, Wozniak et al. (20) observed that rats trained on a position habit reversal task following 0.1 mg/kg MK-801 were capable of learning within a given session but demonstrated a lack of retention when tested on the following day.

Although the findings of the present study agree with previous reports of spared within-session spatial learning, the methods used in the present study deviate from those used by the above authors in one important regard. In the present study, subjects were required to learn to navigate to a new spatial location during each day of training and testing. In contrast, the procedures used by Morris (11) and Kesner and Dakis (8) required subjects to learn to navigate to a spatial location that remained constant over days. Thus, the present study provides a true assessment of the effects of NMDA receptor blockade on the acquisition of novel spatial information within individual sessions.

In summary, only the highest dose of MK-801 used in the present study (0.15 mg/kg) impaired spatial working memory. In contrast to the effects of MK-801 on spatial learning when learning is measured across days (9,14), no dose of MK-801 disrupted within-session spatial learning in the present study. Thus, when coupled with previous research, the findings of the present study further suggest that the NMDA receptor plays a role in the long-term, but not the short-term, storage of spatial information.

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