Posttraining Injections of MK-801 Produce a Time-Dependent Impairment of Memory in Two Water Maze Tasks

Mark G. Packard*+ and Lisa A. Teather†
*Department of Psychology, University of New Orleans, and †Neuroscience Center of Excellence, Louisiana State University Medical School, New Orleans, Louisiana 70148

The role of glutamatergic N-methyl-D-aspartate (NMDA) receptors in memory storage processes was examined using systemic posttraining injections of MK-801. Male Long-Evans rats received an eight-trial (30-s ITI) training session on a spatial or cued water maze task. In the spatial task, a submerged escape platform was located in the same quadrant of the maze on each trial. In the cued task, a visible escape platform was located in a different quadrant of the maze on each trial. Following Trial 8 in both tasks, the rats received a posttraining intraperitoneal injection of the NMDA receptor antagonist MK-801 (0.025, 0.05, 0.1, or 0.2 mg/kg) or saline. On a retention test session 24 h later, latency to mount the escape platform was used as a measure of memory. In both tasks, the retention test escape latencies of animals given MK-801 (0.05 and 0.1 mg/kg) were significantly higher than those of saline-injected controls, indicating a drug-induced impairment of memory. Injections of MK-801 (0.05 mg/kg) did not affect retention when administered 2 h posttraining in either task, indicating that the effects of MK-801 on retention are not due to an influence on non-mnemonic factors. Control experiments indicated that the memory impairing effects of MK-801 were due to an influence on memory for the type of discrimination training given (i.e., spatial or cued) and not due to an influence on a mnemonic strategy common to both tasks. The findings indicate a time-dependent role for NMDA receptor function in memory storage processes.

INTRODUCTION

Investigation of the role of the glutamate N-methyl-D-aspartate (NMDA) receptor in learning and memory processes has been the focus of extensive research, in part due to the well-established role of NMDA receptors in the induction of hippocampal long-term potentiation (LTP), a candidate physiological mechanism underlying memory (for reviews see Collinridge & Bliss, 1993; Maren, 1995). In numerous learning tasks, pretraining systemic administration of the noncompetitive NMDA receptor antagonist MK-801 impairs acquisition (e.g., Heale & Harley, 1990; Ward, Mason, & Abraham, 1990; Pontecorvo, Clissold, White, & Fernkney, 1991; Whishaw & Auer, 1989; Robinson, Crooks, Shinkman, & Gallagher, 1989; Shapiro & Caramanos, 1990; Caramanos & Shapiro, 1994). However, the use of pretraining injections of MK-801 has made it difficult to directly assess the hypothesis that the drug impairs memory processes per se, and some investigators (e.g., Keith & Rudy, 1990; Hargreaves & Cain, 1992) have suggested that MK-801 induced impairments in task acquisition result from non-mnemonic effects on sensorimotor processes (but see also Gallagher, 1990; Morris, 1990; Staubli, 1990).

Posttraining treatments offer a method of differentiating drug effects on memory from those on non-mnemonic factors that may influence task performance. Several posttraining drug and hormone treatments affect memory in a time-dependent manner, losing effectiveness as the interval between training and treatment is extended. This time-dependent nature suggests that any effect on retention produced by an immediate posttraining injection is not due to a proactive effect of the drug on motivational, attentional, sensory, or motoric processes (McGaugh, 1966, 1973, 1989; Gold & McGaugh, 1975; Martinez, 1986).

To our knowledge, relatively few studies have examined the effects of systemic posttraining injections of MK-801 on memory, and the findings have been equivocal. Posttraining MK-801 had no effect...
on retention in a spatial water maze task in gerbils (Mondadori, Weiskrantz, Buerki, Petschke, & Fagg, 1989) and rats (Robinson et al., 1989). However, other studies employing avoidance learning in mice have variously reported memory enhancing (Mondadori et al., 1989) and memory impairing effects of posttraining MK-801 (Mele, Castellano, Cestari, & Oliverio, 1995; Castellano, Mele, & Oliverio, 1996). Several methodological factors including the use of different tasks, training-retention test intervals, doses of MK-801, and species of animals may account for these apparently discrepant findings. In addition, in experiments in which posttraining MK-801 injections impaired memory (Mele et al., 1995; Castellano, Mele, & Oliverio, 1996) the time-dependent nature of this effect was not examined, and thus proactive drug effects on retention test performance cannot be excluded.

The present experiments were designed to further investigate the effects of systemic posttraining administration of MK-801 on memory using spatial and cued water maze tasks. The spatial task requires rats to learn to swim to a hidden escape platform that is positioned in the same spatial location on every trial. The cued task requires rats to learn to swim to a visible escape platform that is positioned in a different spatial location on every trial. Retention in both water maze tasks is enhanced by posttraining systemic (Packard & McGaugh, 1994) and intracerebral (Packard, Cahill, & McGaugh, 1994) injection of the indirect catecholamine agonist d-amphetamine. We examined the effects of posttraining MK-801 in these two tasks using an extensive dose range (0.025–0.2 mg/kg). In addition, after evaluating the effects of the immediate posttraining injections, we examined the time-dependent nature of MK-801 on retention.

**EXPERIMENT 1**

**Methods**

**Subjects.** Subjects were 158 male Long-Evans rats (275–300 g). They were individually housed in a temperature-controlled environment on a 12-h light/dark cycle with the lights on from 7 AM to 7 PM with ad lib. access to food and water. Behavioral testing occurred between 1 and 4 PM.

**Apparatus.** The water maze was a black circular tank 6 ft in diameter and 1.5 ft in height. The tank was filled with water (25°C) to a depth of 20 cm. The maze was located in a room containing many extramaze cues. Four starting positions (north, south, east, west) were equally spaced around the perimeter of the tank, dividing the pool into four equal quadrants. The rectangular Plexiglas escape platform (11 × 14 × 19 cm) was submerged at a depth of 1 cm. For the cued water maze task, a white rubber ball (8 cm diameter) was attached to the top of the submerged platform and protruded above the water surface. The platform could be used as a step to mount the ball and escape the water.

**Drugs.** MK-801, ([+]-10,11-dihydro-5-methyl-5H-dibenzo[a,d]cycloheptene-5,10 imine; Research Biochemicals Int.) was dissolved in physiological saline. Injections (MK-801 doses; 0.025, 0.05, 0.1, or 0.2 mg/kg) were administered intraperitoneally (ip). Injection volume was 1 ml/kg, and control animals were injected ip with an equal volume of saline.

**Behavioral procedures.** The behavioral procedures were similar to those described previously (Packard & McGaugh, 1994; Packard et al., 1994). In both the spatial and cued tasks, the animals received one training session of eight trials (i.e., swims). The animal was placed into the tank facing the wall at one of the four designated start points (N, S, E, and W) and allowed to escape onto the hidden or cued platform. A different starting point was used on each trial such that each starting point was used twice within the eight trials. If an animal did not escape within 60 s, it was manually guided to the escape platform. After mounting the platform, rats remained on the platform for 20 s. Following each trial they were removed from the maze and placed in a holding cage for a 30-s intertrial interval. The latency to mount the escape platform was recorded and used as a measure of acquisition of each task.

In the spatial task, the hidden escape platform was located in the same quadrant on every trial. In the cued task, the escape platform was placed in a different quadrant on each trial such that each of the four quadrants contained the escape platform on two of the eight trials. The locations of the start points for the cued task were arranged so that distance to the escape platform (i.e., proximal or distal) and location of the platform relative to the start point (i.e., left or right) were counterbalanced across the eight trials.

Immediately following the last training trial animals were assigned to treatment groups and received a posttraining ip injection of either MK-801 or saline. Rats received one of four doses of MK-801 or saline (spatial task: saline, n = 17; 0.025 mg/kg, n = 17; 0.05 mg/kg, n = 15; 0.1 mg/kg, n = 20; 0.2 mg/kg, n = 20; cued task: saline, n = 16; 0.025 mg/
Retention was tested 24 h after completion of the training. The retention test consisted of two trials, and as during training a 20-s stay on the platform and a 30-s ITI were used between trials. For the spatial task the submerged escape platform was located in the same quadrant of the maze as during training. On each of these two trials a different starting point located distal to the escape platform was used. For the cued task the visible escape platform was placed in a different quadrant of the maze on each trial, and a different starting point located distal to the escape platform was used. In both tasks the latency to mount the escape platform was recorded on the retention test trials and used as a measure of memory for the previous day’s training session.

Delayed posttraining injections. Additional groups of animals received identical training in either the spatial or the cued task but were injected with MK-801 (0.05 mg/kg; n = 8 per group) or saline (n = 8 per group) 2 h after training. The delayed injection groups were run to examine whether the effects on retention observed with the immediate injections were due to a proactive effect of MK-801 on retention test performance. Thus, the dose used for the delayed injections (0.05 mg/kg) was chosen after evaluating the effectiveness of the immediate posttraining injections.

Results

Spatial water maze task. The training day escape latencies (i.e., prior to injections) for the spatial task are illustrated in Fig. 1A. A two-way one repeated measure ANOVA computed on the escape latencies on the training day (i.e., prior to the post-training injections) revealed no significant Group differences [F(4, 84) = .928, n.s.]. A significant Trial effect [F(4, 7) = 104.7, p < .01] indicated that all groups improved over the eight training trials, obtaining mean escape latencies of 10–15 s on Trials 7 and 8 (Fig. 1A).

The effect of posttraining administration of MK-801 on retention in the spatial task is shown in Fig. 1B. A two-way one repeated measure ANOVA computed on the escape latencies revealed a significant Group X Trial interaction [F(4, 84) = 3.40, p < .05], a significant Group effect [F(4, 84) = 9.57, p < .01], and a significant Trial effect [F(1, 4) = 192.5, p < .01]. Tests of simple main effects (Group within Trial) revealed a significant group effect on retention test trial one [F(4, 84) = 10.82, p < .01]. Scheffe post-hoc tests showed that the latencies of rats receiving MK-801 at a dose of 0.05 mg/kg (F = 3.08, p < .05) and 0.1 mg/kg (F = 4.95, p < .05) were significantly higher than those of the saline treated rats on Trial 1, indicating an impairment in memory. A similar test of main effects also revealed a significant Group difference on the second retention test trial [F(4, 84) = 4.58, p < .01]. Scheffe post-hoc tests showed that the latencies of rats receiving MK-801 at a dose of 0.05 mg/kg (F = 3.22, p < .05) and 0.1 mg/kg (F = 3.89, p < .05) were significantly higher than those of the saline-treated rats on Trial 2.

The retention test escape latencies of rats that received injections of MK-801 (0.05 mg/kg) delayed 2 h postraining were not significantly different
45MK-801 AND MEMORY

801 on retention in the cued task is shown in Fig. 2B. A two-way one repeated measure ANOVA computed on the escape latencies revealed a significant Group X Trial interaction \[ \text{F}(4, 72) = 4.97, p < .01 \], a significant Group effect \[ \text{F}(4, 72) = 2.81, p < .05 \], and a significant Trial effect \[ \text{F}(1, 4) = 85.93, p < .01 \]. Tests of simple main effects (Group within Trial) revealed a significant group effect on retention test Trial 1 \[ \text{F}(4, 72) = 4.05, p < .01 \]. Scheffe post-hoc tests showed that the latencies of rats receiving MK-801 at a dose of 0.05 mg/kg \( (F = 2.51, p < .05) \) and 0.1 mg/kg \( (F = 3.20, p < .05) \) were significantly higher than those of the saline-treated rats on Trial 1, indicating an impairment in memory. A similar test of main effects revealed no significant Group difference on the second retention test trial \[ \text{F}(4, 72) = 1.58, \text{n.s.} \].

The retention test escape latencies of rats that received injections of MK-801 (0.05 mg/kg) delayed 2 h posttraining were not significantly different than those of saline-treated rats \( \text{F}(1, 23) = .87, \text{n.s.} \).

EXPERIMENT 2

Although the findings of Experiment 1 indicate that posttraining administration of MK-801 impairs memory in both water maze tasks, the effective doses were the same in the two tasks (0.05 and 0.1 mg/kg). This raises the possibility that the MK-801-induced impairment in retention may have been due to an effect on memory for a general “search strategy” common to both tasks. For example, in both tasks the escape platform was located in a position the same distance from the pool wall; in the spatial task this position was constant on each trial, and in the cued task this position was changed on each trial. Therefore, in either task MK-801 may have impaired memory for a strategy such as “swim X distance from the pool wall.” Alternatively, MK-801 may have impaired memory for the “type” of discrimination training (i.e., spatial or cued) used. These two hypotheses can be assessed by testing animals on the opposite version of the task in which they are trained (Packard & McGaugh, 1994). If the memory impairing effects of MK-801 were due to an influence on memory for a search strategy common to both tasks, then such impairment should be observed regardless of the type of training (i.e., spatial or cued) received. Alternatively, if the memory impairing effects of MK-801 were due to an influence on memory for the type of training received, then no impairment should be observed if rats are tested on the task different than that they were trained on. Therefore, in Experiment 2 rats received training on either the
spatial or the cued task, followed by posttraining administration of MK-801 or saline. On a retention test session 24 h later, the rats trained in the cued task were tested in the spatial task, and rats trained in the spatial task were tested in the cued task.

Methods

Subjects. The subjects were 32 male Long–Evans rats (275–300 g) housed under conditions identical to those of Experiment 1.

Apparatus. The apparatus was the same as that used in Experiment 1.

Drugs. MK-801 (Research Biochemicals Int.) was dissolved in physiological saline. Drug administration procedures were identical to those of Experiment 1. A dose of 0.05 mg/kg was used, based on its effectiveness in impairing memory in a time-dependent manner in both tasks in Experiment 1.

Behavioral procedures. The training procedures were similar to those described for Experiment 1. Immediately following an eight-trial training session in either the spatial or the cued tasks, rats received a posttraining injection of MK-801 (0.05 mg/kg; n = 8 per group) or saline (n = 8 per group). Twenty-four hours later, animals trained in the spatial task were given an eight-trial retention test in the cued task. Animals trained in the cued task were given an eight-trial retention test in the spatial task. The retention test procedures were identical to those of Experiment 1 (i.e., 60-s maximum swim/guided to platform, 20-s stay on platform, 30-s ITI).

Results

The retention test escape latencies of saline- and MK-801-treated animals trained on the spatial task and tested on the cued task are shown in Fig. 3A (solid lines). The retention test escape latencies of saline- and MK-801-treated animals trained on the cued task and tested on the spatial task are shown in Fig. 3B (solid lines). Two-way one repeated measure ANOVAs revealed no significant Group differences between the retention test escape latencies of saline- and MK-801-treated animals under either condition (Fig. 3A; F(1, 14) = .04, n.s., Fig. 3B; F(1, 14) = .005, n.s.). Thus, the memory impairment produced by posttraining MK-801 on both discrimination tasks (Experiment 1) was not observed when animals were tested in tasks different from that in which they received original training. For purposes of comparison, the training day escape latencies of saline animals from Experiment 1 in the cued and spatial tasks are also shown in Figs. 3A and 3B, respectively (hatched lines). Visual comparison of the performance of these groups to that of animals tested in the task different from that in which they were originally trained shows that the latter animals received little benefit from a single eight-trial training session in either task (Figs. 3A, and 3B).
DISCUSSION

Previous findings indicate that posttraining MK-801 injections in gerbils (Mondadori et al., 1989) and rats (0.1 mg/kg; Robinson et al., 1989) have no effect on retention in a spatial water maze task. These findings appear to contrast with the memory impairing effect of posttraining MK-801 (0.1 mg/kg) observed in the spatial task in the present study. One aspect of the analysis of retention in the spatial water maze task may be relevant in reconciling these apparently discrepant results. Specifically, in the present study the memory impairing effects of MK-801 in both water maze tasks were observed primarily on the first retention test trial. We suggest that the initial trial provides the most sensitive measure of memory for the previous day's training and hence is the most appropriate trial on which to examine drug influences on memory. However, the first retention test trial provides exposure to the aversive nature of the water and the spatial location of the escape platform. Moreover, as during training, rats that failed to escape on the first retention trial in 60 s were guided to the platform and remained there for 20 s. These factors may contribute to the apparent “recovery” of memory observed in drug-treated rats. Analysis of escape latencies over a block of several retention test trials (i.e., Robinson et al., 1989, escape latencies not shown) may therefore not be likely to reveal a drug-induced memory impairment.

The present results are consistent with previous findings indicating that posttraining MK-801 administration impairs memory storage processes in inhibitory avoidance tasks (Mele et al., 1995; Castellano et al., 1996). Findings of studies using intracerebral injections of the competitive NMDA receptor antagonist AP5 are also consistent with the hypothesis that NMDA receptor blockade produces memory impairment. For example, posttraining intracerebroventricular (Flood, Baker, & Davis, 1990) and intra-amygdala (Izquierdo, Cunha, Rosat, Jerusalinsky, Ferreira, & Medina, 1992; Liang, Hon, & Davis, 1994) injections of AP5 impair memory for inhibitory avoidance training.

The present study does not reveal the mechanism by which posttraining administration of MK-801 impairs memory. However, much of the impetus for examining the effects of NMDA receptor antagonists on memory has been derived from the well-established role of NMDA receptors in the induction of LTP, a putative physiological mechanism underlying memory (e.g., Collinridge & Bliss, 1993; Maren, 1995). While pharmacological evidence indicates a selective role for NMDA receptors in the induction of LTP, the precise time point during training at which an analogous "tetanized"
state has been attained by an intact animal in a given learning task is unknown. Nonetheless, it seems unlikely that post-training blockade of NMDA receptors by MK-801 would affect an LTP induction process, particularly in view of evidence that a high dose of MK-801 (1.0 mg/kg) fails to block LTP unless the drug is injected 150 min prior to tetanization (Abraham & Mason, 1988). However, within the LTP-memory framework, it is conceivable that posttraining blockade of NMDA receptors may affect an LTP-linked memory mechanism by blocking subsequent cell signaling cascades (e.g., release of retrograde messengers) dependent on the intracellular calcium increase that results from NMDA receptor activation.

As an alternative to the LTP-memory framework, it is possible that posttraining blockade of NMDA receptors may affect memory via interactions with other neurotransmitter systems implicated in posttraining memory processes. For example, glutamate modulates the release of acetylcholine (Giovannini et al., 1995; Login et al., 1995), norepinephrine (Navarro, Cabrena, & Donoso, 1995), and dopamine (Krebs et al., 1991; Whitten, Maione, Biggs, & Fowler, 1994). Interactions between dopaminergic and glutamatergic systems may be particularly relevant in view of evidence that postraining injections of the dopamine D2 receptor agonist quinpirole enhances memory in both spatial and cued water maze tasks (Packard & McGaugh, 1994), and quinpirole attenuates the memory impairing effect of postraining injection of the competitive NMDA receptor antagonist CPP (Mele, Castellano, Cestari, & Oliverio, 1995).

One finding of early studies examining the effects of NMDA receptor antagonists (e.g., AP5, MK-801) on acquisition of different learning tasks is that these drugs produced a “task-dependent” pattern of acquisition impairment that was similar to that observed following hippocampal system lesions (e.g., Morris, Anderson, Lynch, & Baudry, 1986; Robinson et al., 1989; Staubli, Thibault, DiLorenzo, & Lynch, 1989). For example, chronic postraining intracerebroventricular injections of AP5 impaired acquisition of the spatial but not the cued water maze task (Morris et al., 1986; but see also Cain, Saucier, Hall, Hargreaves, & Boon, 1996). However, in the present study, the effect of acute postraining MK-801 administration was not task specific, as retention in both water maze tasks was impaired. In addition, a considerable literature now exists that appears incompatible with the hypothesis that the effects of NMDA receptor blockade are confined to hippocampal-dependent memory tasks. For example, the NMDA receptor antagonist CPP impairs acquisition of non-hippocampal-dependent cued learning in both appetitive (Lyford & Jarrard, 1991) and aversively motivated learning tasks (Upchurch & Wehner, 1990). Other examples include the impairing effect of AP5 on amygdala-dependent emotional memory processes (Izquierdo et al., 1992; Kim and McGaugh, 1992; Liang, Hon, & Davis, 1994) and MK-801 on caudate nucleus-dependent (Packard & McGaugh, 1992; McDonald & White, 1994) cued water maze behavior (present study). Thus, increasing evidence indicates a role for NMDA receptor function in both hippocampal and non-hippocampal-dependent memory processes.

The hypothesis that NMDA receptors mediate the acquisition of different “forms” of memory is also of interest in view of evidence from both brain lesion (Packard, Hirsh, & White, 1989; Kesner, Bolland, & Dakis, 1993; McDonald & White, 1993) and postraining intracerebral drug injection studies (Packard & White, 1991; Packard et al., 1994; Packard & McGaugh, 1996) indicating that the hippocampus and the caudate nucleus are parts of independent memory systems. In particular, evidence suggests that the hippocampus and the caudate nucleus may selectively mediate the acquisition of spatial and cued water maze behavior, respectively (Morris et al., 1982; Sutherland, Whishaw & Kolb, 1983; Packard & McGaugh, 1992). The hippocampus and the caudate nucleus contain high and moderate densities of NMDA receptors, respectively (for review see Ottersen, Hjelle, Osen, & Laake, 1995), and a systemic injection of MK-801 would likely influence glutamatergic transmission in each of these memory systems. Thus, the memory impairing effects of postraining MK-801 in the spatial task may have been due to a blockade of hippocampal NMDA receptors, while blockade of NMDA receptors in the caudate nucleus may have mediated the memory impairing effects of postraining MK-801 in the cued task. This hypothesis is consistent with our recent finding that postraining intracerebral injections of the competitive NMDA receptor antagonist AP5 produces a selective impairment in these two water maze tasks; intrahippocampal injections selectively impair memory in the spatial task, while intracaudate injections selectively impair memory in the cued task (Packard & Teather, in press). The ability of intracerebral injections of NMDA receptor antagonists into selective brain regions to influence memory suggests a widespread role for glutamatergic neurotransmission in memory. However, within a given brain struct-
ture, this role may be dictated by the type of memory required for task acquisition.

REFERENCES


