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MK-801 Impedes the Acquisition of a Spatial Memory Task in Rats

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McLAMB, R. L., L. R. WILLIAMS, K. P. NANRY, W. A. WILSON AND H. A. TILSON. *MK-801 impedes the acquisition of a spatial memory task in rats.* PHARMACOL BIOCHEM BEHAV **37**(1) 41–45, 1990.—Several studies have reported that MK-801 impairs the acquisition of various learning and memory tasks, while others suggest that MK-801 may interfere with performance rather than having a specific effect on memory. To characterize further the effects of MK-801 on learning and memory, MK-801 (0.05 mg/kg, SC) was administered prior to or immediately after learning trials in a trial-independent water maze task. Since MK-801 may affect nonassociative variables that may influence learning and memory, motor activity and general reactivity measures were also determined for 0.0125, 0.025, or 0.05 mg/kg of MK-801 administered SC. Since MK-801 may also be used to treat children with epilepsy, we investigated the possible persistent cognitive effects on neonates. MK-801 (0.02 mg/kg, SC) was administered at postnatal days 9–15 and tested in the same task as above starting at day 36 of age. There were no persistent effects of neonatal treatment. However, in adult rats, MK-801 impaired the acquisition of the water maze task but did not affect performance during a recall task in the same apparatus. At doses affecting learning, there were no effects on motor activity or general reactivity in adult rats. These results are consistent with the conclusion that MK-801 interferes with acquisition of spatial learning in the rat.

MK-801 Learning and memory Rats Water maze acquisition

MK-801, (+)-5-methyl-10,11-dihydroxy-5h-dibenzo(a,d)cyclohepten-5,10-imine, is a selective, noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist that blocks NMDA-induced excitation by interacting with open ion channels linked to the NMDA receptor (16). Clinically, MK-801 has been noted for its anticonvulsant potential, although some patients have reported side effects including mild tenseness, anxiety, dizziness, and difficulty in concentration (22). MK-801 can reduce or block seizure activity and reduce the severity of behavioral impairment and neuronal cell loss from damage produced by centrally administered NMDA (18).

It is known that glutamate receptor antagonists can block the development of long-term potentiation (12), a form of synaptic plasticity induced by high frequency afferent stimulation (11). Intracerebroventricular infusion of aminophosphonovaleric acid (AP5), a competitive NMDA receptor antagonist, has been reported to impair place learning (12), acquisition of a radial arm maze (4), retention of a step-through passive avoidance task (4) and acquisition of an olfactory discrimination task (20). Recently, it was reported that MK-801 interfered with acquisition of a passive avoidance task (1,23). Robinson *et al.* (17) reported that MK-801 impaired place, but not cue learning in a water maze task at 0.05 mg/kg, while interfering with both at a higher dose (0.08 mg/kg). Recently, Whishaw and Auer (24) reported that MK-801 impaired acquisition of a new place response in a water maze, while having no effect on performance of a new cue response or a well learned place response. However, Wozniak *et al.* (27) concluded that MK-801 did not interfere with the ability to learn, but may have impaired recall of a learned event, while Pontecorvo and Clissold (15) reported that MK-801 would result in disorientation leading to poor performance rather than a specific effect on memory.

The purpose of the following experiments was to characterize in greater detail the effects of MK-801 on learning in the rat. In these studies, MK-801 was administered prior to or immediately after learning trials in a trial-independent water maze task. Since MK-801 might affect performance or nonassociative variables that can influence learning and memory, effects of MK-801 on motor activity and general reactivity were also determined. Drug-induced changes in either measure could influence the rate of acquisition.

Previous work (14) found that prenatal exposure to phencyclidine, which is a NMDA antagonist, interfered with several developmental milestones in rats. However, these effects were observed at doses having maternal and fetotoxicity. Recently, it

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was reported that MK-801 given postnatally interfered with olfactory discrimination learning (19). Possible persistent effects of MK-801 exposure on learning have not been investigated. In the present study, MK-801 was given repeatedly during postnatal development in order to block NMDA receptors during the postnatal period of maturation of glutamergic/aspartamergic structures (3,26). Possible persistent effects on learning were determined at least two weeks after the last dose of MK-801.

METHOD

Adult Animals

Animals were housed in a temperature- (20 ± 2°C) and humidity- (50 ± 10%) controlled colony room with a constant 12-hr light/dark cycle (lights on at 0700 hr). Both laboratory chow (NIH diet No. 31) and tap water were continuously available. Male Fischer-344 rats (Charles River Breeders, Raleigh, NC) weighing 275–300 g were housed four per cage.

Dose-response curves for adults were determined for both 1-hr activity levels and for acoustic startle response. In both measures animals were dosed with either saline, 0.0125, 0.025, or 0.05 mg/kg MK-801 SC 1 hr prior to being placed in the test chamber. A dose of 0.1 mg/kg was also tested in the activity chambers; however, due to obvious impairments in motor function, this dose was not used in learning studies. The acquisition of a spatial navigation task was investigated over eight days using saline or 0.05 mg/kg MK-801 SC 1 hr prior to testing. To determine if any observed differences were due to an impairment in learning or in the consolidation of information, another group of animals were tested in the water maze with injections (saline or 0.05 mg/kg MK-801 SC) given immediately following daily test sessions.

Neonates

For the neonatal study, time-bred Fischer-344 dams from Charles River Breeders were used. On day 1 (one day postpartum), all the rat pups were pooled and cross-fostered with three dams receiving 4 male and 4 female pups and one dam receiving 2 male and 5 female pups. On days 9–15 all pups were injected SC with either saline or 0.2 mg/kg MK-801 dissolved in saline. Each litter contained an equal number of control and MK-801-treated rats. The pups were weaned and housed four per cage on day 22 with only the males being used in subsequent behavioral testing. Males were used in order to be consistent with previous work in the experiment with adult animals.

Neonates were tested for the acquisition of a spatial navigation task starting on day 36. Animals were given 9 daily sessions followed by a single posttraining retention trial ("free swim"). On day 47 animals were tested for motor activity levels for 1 hr in the motor activity chambers. Acoustic startle response was measured on day 52.

Behavioral Procedures

The acquisition of a spatial navigation task was examined using a Morris water maze (12), as modified by Mundy and Tilson (13). Briefly, animals were trained to swim to a platform hidden in a large circular pool (148 cm diameter × 60 cm high) located in a test room containing numerous extramaze cues. The pool was filled to a depth of 40 cm with water (28 ± 2°C) made opaque with powdered milk. The transparent platform was 10 cm in diameter and submerged 1.5 cm below the surface. Four equally spaced points around the edge of the pool were used as start points and divided the pool into four equal quadrants.

Daily sessions consisted of 4 trials, with approximately 5 minutes between each trial. Each rat was placed in the maze at one of the four starting points, which were used only once per day in a pseudorandom sequence. The platform was fixed in the center of one of the four quadrants and remained in that location for the duration of training for each rat. The escape latency (the time taken to locate the platform) and crossover rate (the number of times an animal crossed into a new quadrant divided by the duration, in seconds, of the trial) were recorded up to a maximum of 60 sec. If a rat did not locate the platform within that time, it was placed on the platform for 15 sec and an escape latency of 60 sec was recorded for that trial. A daily mean escape latency and daily mean crossover rate were determined by taking the average of the four trials for each animal.

To determine the extent of spatial learning, the final test consisted of one 60 sec retention test ("free swim"), during which the platform was removed from the pool. The time spent swimming in each of the four quadrants was recorded for each rat.

To determine acoustic startle response, animals were placed in a small acrylic cage ($13.5 \times 25 \times 9$ cm) suspended 1 mm above a platform attached to a load cell assembly. The test cage was housed in a sound- and light-attenuating chamber (Model AC-2, Industrial Acoustic Co., Bronx, NY). The rats were exposed to 20 trials of a 110 dB, 200 msec, 8 kHz tone from a speaker suspended 27.5 mm above the test cage. The magnitude of the startle response was quantified by a peak hold circuit which measured the most intense response occurring over the first 100-msec period after each stimulus presentation. A PDP-11 minicomputer using Super-Sked software performed the analog/digital transformation of the transducer output. An average response was obtained for each subject by determining the mean for all 20 trials.

Motor activity was recorded in four identical rectangular Plexiglas chambers ($43 \times 21 \times 18$ cm) equipped with two parallel rows of photocells, 20 per side, positioned either 5.5 or 13 cm along the long axis of the chamber. Interruption of the photobeam was detected by a PDP-11 minicomputer, recorded as an activity count, and stored for later analysis (21).

Statistical Analysis

A repeated measures analysis of variance (ANOVA) (25) was used to determine overall effects of treatment or interactions of treatment with repeated testing followed by a Greenhouse-Geisser adjustment for degrees of freedom for all repeated measures factors and interactions with repeated factors to compensate for the lack of specificity (6,7). In cases where repeated testing was not a factor, a one-way ANOVA was used to determine significant treatment effects. Post hoc comparisons between groups were made using Fisher's Least Significance Test. The accepted level of significance was $p<0.05$.

RESULTS

Adult Animals

The SC administration of MK-801 had no consistent effect on total spontaneous motor activity of adult rats at doses of 0.0125 to 0.05 mg/kg, $F(3,31)=1.05$ (Fig. 1A). MK-801 also had no significant effect on the acoustic startle response, $F(3,31)=0.34$ (Fig. 1B). Injecting MK-801 prior to training in the water maze interfered with the time to find the platform (Fig. 2A). Repeated measures ANOVA on the latency data indicated significant treatment, $F(1,12)=11.23$, $p<0.0058$, and day, $F(7,84)=24.51$, $p<0.0001$, effects. The treatment by day interaction was not statistically significant, $F(1,12)=2.13$, $p>0.0998$. There was a significant day effect, $F(7,84)=7.12$, $p<0.0001$, but no significant treatment, $F(1,12)=2.44$, $p>0.14$, nor day by treatment

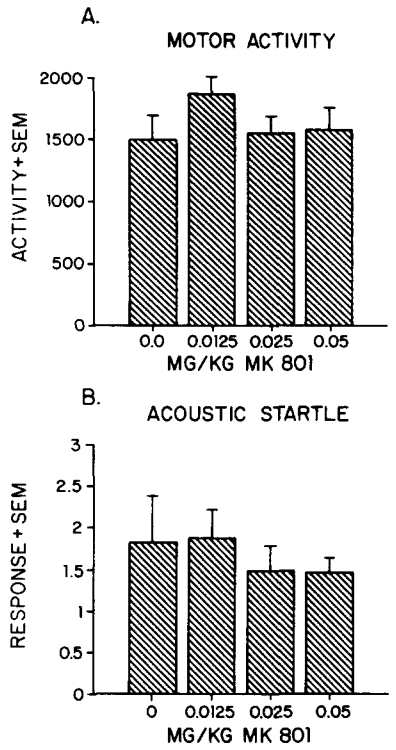

FIG. 1. Effects of SC administration of MK-801 on sensorimotor function of adult rats. Rats were dosed with 0, 0.0125, 0.025 or 0.05 mg/kg and tested one hour later. (A) Data are average activity counts per 60 min ±S.E. for 8 rats per group. (B) Data are average volts ±S.E. for 8 rats.

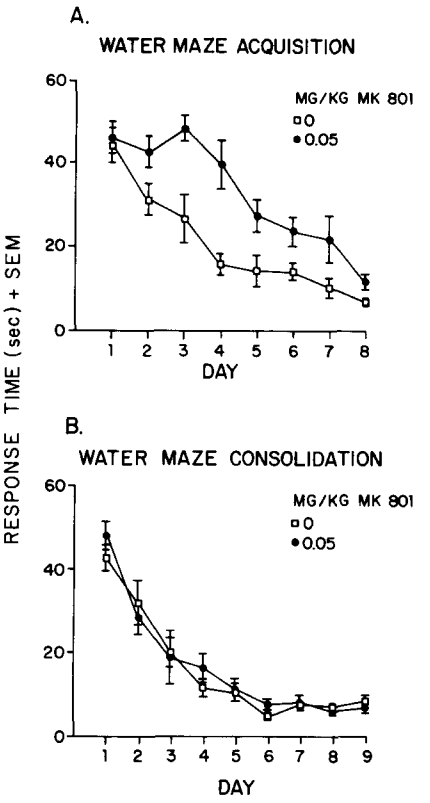

FIG. 2. Effects of MK-801 given before (A) or immediately after (B) daily training trials in the water maze. There were 8 rats per group and data are average latencies (sec) ±S.E. to find the platform.

interaction, $F(7,84)=1.59$, $p>0.15$, on crossover rate as indicated by a repeated measures ANOVA. When tested for retention ("free swim") after 8 days of training, rats previously treated with MK-801 spent 31.4±3.9 sec in the training quadrant, while control animals spent 29.5±3.4 sec, $t(14)=0.27$, $p>0.7166$, two-tailed.

When injected immediately after each day of training, MK-801 had no significant effect on the rate of acquisition (Fig. 2B). Repeated measures ANOVA indicated no significant treatment effect, $F(1,14)=0.13$. There was a significant day effect, $F(8,112)=46.52$, $p<0.0001$, but the treatment by day interaction was not significant, $F(8,112)=0.58$. There was no effect on retention ("free swim") after 9 days of training, $t(14)<1.0$, nor were there effects on the crossover rate at any time during the experiment.

Neonates

The neonatal administration of 0.2 mg/kg on days 9–15 produced a transient 10–15% decrease in body weight during dosing. By the day of testing on day 36, the body weights of MK-801-pretreated rats were not different from controls. Neonatal administration of MK-801 had no significant effect on subsequent motor activity, $F(1,12)=0.99$, or startle responsiveness, $F(1,12)=1.85$ (Table 1). In the water maze (Fig. 3), a repeated measures ANOVA indicated that neonatal administration of MK-801 had no significant effect, $F(1,11)=0.11$, nor did treatment interact with day, $F(8,88)=0.79$. In the posttraining test ("free swim"), the MK-801 animals spent 36.0±3.0 sec in the training quadrant, while the controls spent 29.1±2.9 sec, $t(11)=1.62$, $p>0.1336$, two-tailed. MK-801 treatment had no effect on the crossover rate at any time during acquisition or retest.

DISCUSSION

When given systemically to adult animals, the noncompetitive NMDA receptor antagonist MK-801 significantly interfered with the rate of acquisition in a place-navigation task in the water maze. These effects occurred following a dose (0.05 mg/kg, SC) which had no significant effect on crossover rate in the water maze, spontaneous motor activity or reactivity to an acoustic startle stimulus. In addition, administration of MK-801 immediately after daily training trials in the water maze had no significant effect on responding on the next day, suggesting that MK-801 has little effect on the process of consolidation.

TABLE 1

MOTOR ACTIVITY AND ACOUSTIC STARTLE RESPONSIVENESS OF RATS EXPOSED NEONATALLY TO MK-801

	Average Response ± S.E.*	
Treatment	Motor Activity (counts/60 min)	Startle Response (volts)
Control	2131 ± 223	0.84 ± 0.10
MK-801	1828 ± 201	1.05 ± 0.11

*There were 7 rats in the control group and 6 in the MK-801 group.

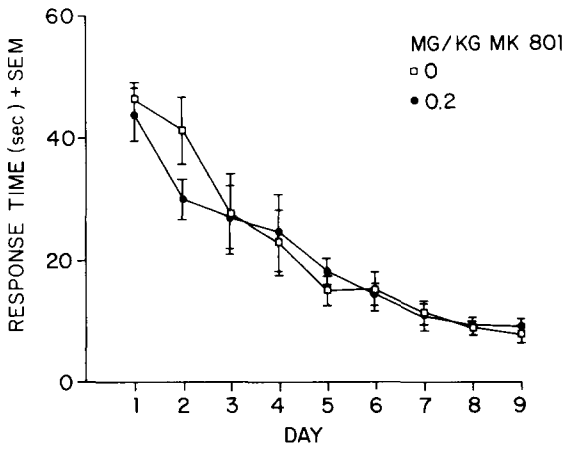

FIG. 3. Effects of neonatal exposure to MK-801 on subsequent learning in the water maze. Neonates were dosed with 0.2 mg/kg, SC, on days 9–15 postnatally and tested in the water maze starting on day 36. Data are average latencies (sec) to escape ± S.E. There were 7 rats in the control group and 6 in the MK-801 group.

There is increasing evidence that MK-801 might interact directly with binding sites for phencyclidine (10). Koek *et al.* (9) reported that MK-801 produced phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. Furthermore, Koek *et al.* (8) suggested that phencyclidine-like drugs mediate their behavioral effects by reducing neurotransmission at excitatory amino acid synapses utilizing NMDA-preferring receptors. Since phencyclidine has been reported to impair learning and memory (5), it was predicted that MK-801 might have similar effects on cognitive function.

The results of our experiments support the findings of others that MK-801 interferes with the rate of acquisition of a spatial task (17,24). These results also support the conclusion of Wozniak *et al.* (27) that rats treated with MK-801 are capable of learning. When injected prior to acquisition, MK-801-treated rats eventually learned the task, reaching asymptotic levels of performance within 8 training days. In addition, when given a retention training test ("free swim"), in the absence of MK-801, there were no differences between groups. Our explanation for these findings is that MK-801-treated rats solved the task by using a learning strategy not dependent upon NMDA-mediated mechanisms.

Pontecorvo and Clissold (15) used a two-choice continuous nonmatching to sample working memory task in rats and found that MK-801 reduced accuracy at doses that did not produce a reduction in response probability. Since the reduction in accuracy was associated with an increase in false alarms, it was concluded that MK-801 may produce a state of disorientation rather than having a specific effect on working memory. Our data suggest that in the water maze task, rats may not be disoriented. The dose of MK-801 affecting water maze acquisition had no effect on other measures of sensorimotor function, i.e., motor activity and startle responsiveness and did not affect crossover rates in the maze.

The detrimental effects of MK-801 on learning in the present study were evident only if the agent was given prior to testing. This observation is consistent with the finding that MK-801 produces a posttraining effect only at doses that are 10 times those required to produce a pretraining effect on passive avoidance learning in mice (2).

Another important finding of these experiments is that neonatal treatment with MK-801 appeared to have little or no persistent effect on sensorimotor function or learning ability. Stanton and Jensen (19) have recently reported that administration of MK-801 interfered with the acquisition of an olfactory discrimination task in 16-day-old rats. In our experiments, which used a relatively high dose of MK-801 (0.2 mg/kg) resulting in a transient loss of weight, we observed no persistent effects two weeks after the last dose of MK-801.

The postnatal administration of MK-801 had no long-lasting effect on the ability to learn a water maze task or on measures of sensorimotor function. Dosing of MK-801 occurred during postnatal development of glutaminergic/aspartamergic structures (3,26), producing a transient loss of body weight. These observations are different from those of Nabeshima *et al.* (14), who found postnatal neurological effects of prenatal PCP exposure at doses causing maternal body weight loss and fetotoxicity, including decreased fetal body weight and length, decreased viability and decreased body weight during nursing. In addition, Stanton and Jensen (19) reported that a single injection of MK-801 to 16-day-old rat pups interfered with learning of an olfactory discrimination task when tested within 40–70 min after dosing. Our data indicate that such effects on learning capabilities may be transient since no effects on learning were observed three weeks or more after cessation of dosing.

In summary, systemic administration of MK-801 to adult rats interfered with the rate of learning of a spatial memory task in the water maze. However, MK-801-treated rats eventually learned the task and exhibited no significant impairment when tested 24 hours after the last training trial. Additional studies are needed to elucidate the mechanism by which blockade of NMDA receptors can interfere with spatial learning.

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