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MK-801 and AP5 Impair Acquisition, But Not Retention, of the Morris Milk Maze

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HEALE, V. AND C. HARLEY. *MK-801 and AP5 impair acquisition, but not retention, of the Morris milk maze*. PHARMACOL BIOCHEM BEHAV **36**(1) 145–149, 1990.—The effects of the NMDA blockers, AP5 and MK-801, were assessed in two spatial tests. AP5 (10 μg in 2 μl ICV, N = 6), or MK-801 (0.07 mg/kg IP, N = 6), significantly increased open-field activity in male Long-Evans rats in two 3-min tests (Days 1 and 2) compared to control groups receiving equal volume saline injections (N = 12). In the Morris milk maze, NMDA blockade significantly impaired acquisition performance on two blocks of six trials, which followed each open-field test. Only control animals showed evidence of acquisition on a drug-free retention test assessing latency to reach the expected platform area and number of crossings in the area on Day 4. Retention was tested in control animals under NMDA blockade on Day 6. There was no effect of NMDA blockade on retention in the Morris milk maze. These results support the hypothesis that NMDA receptors are critical for the initiation of synaptic modification underlying place learning, but are not necessary in synaptic transmission during retrieval of place information.

MK-801 Spatial memory Hippocampus AP5 NMDA Activity

THE NMDA receptor has been shown to play a critical role in the induction of long-term potentiation (1). The operation of the synaptic channels coupled to the NMDA receptor which require the conjunction of postsynaptic depolarization and presynaptic transmitter release for their activation, meets the formal requirements of the Hebbian synapse and is a logical candidate for a biological memory or learning mechanism (2, 4, 5). If the NMDA mechanism is critically involved in triggering synaptic modifications during learning, but not in synaptic transmission during information retrieval, it follows that NMDA receptor blockade should impair acquisition, but not interfere with previously acquired memories. Both competitive and noncompetitive blockers of the NMDA receptor/channel complex are available.

The hippocampus has the highest density of NMDA receptors (7), is demonstrably involved in spatial coding at the cellular level (10,11) and is a critical structure for acquisition of the Morris milk maze (8).

In 1986, Morris *et al.* (9) demonstrated that chronic ICV minipump administration of AP5, a specific competitive blocker of the NMDA receptor, could prevent the occurrence of both hippocampal LTP and spatial learning in the Morris milk maze. Visual discrimination learning was unaffected. These results suggested that synapse modification triggered by NMDA receptor activity was critical in the formation of the spatial map memory necessary to acquire the Morris milk maze.

In 1987, however, Halliwell and Morris (3) tested the noncompetitive NMDA blocker, MK-801, in the same task and found no effect on acquisition of the milk maze even at drug levels that produced marked ataxia. Two differences between the earlier AP5 study and the more recent MK-801 study are the differences in route and timing of administration. AP5 was administered as a chronic intracerebroventricular (ICV) infusion over a two-week period during acquisition in the 1986 study, while MK-801 was given as an acute intraperitoneal (IP) injection prior to each training day in the 1987 study. Long-term NMDA blockade may be necessary to prevent the acquisition effect. This would be particularly true if some form of posttraining memory might be available to influence NMDA-dependent processes.

A second explanation for the discrepancy in effects with the two types of NMDA blockade is the use-dependency of noncompetitive NMDA blockers. In studies using brain slices it has been demonstrated that MK-801 is slow in blocking NMDA function until NMDA activation has occurred (13). Prior NMDA activation apparently permits entry of the blocker into the associated ion channel and entry is necessary for blockade of the NMDA channel. If no use of the channel occurs the blocker does not enter. It is possible that adequate use of the channel for effective blockade did not occur prior to milk maze training.

The present study is a further investigation of the role of NMDA receptors in milk maze acquisition. Both MK-801 and

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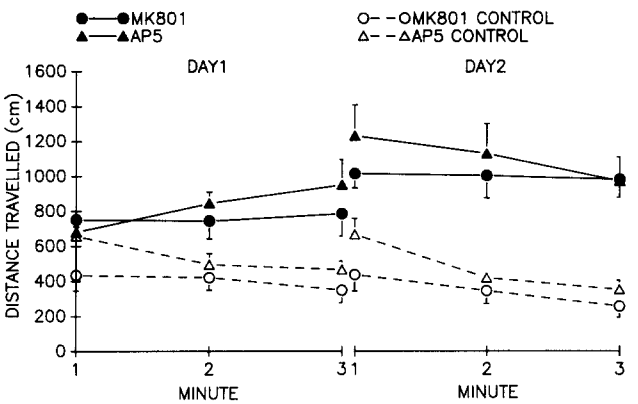

FIG. 1. Mean distance travelled each minute on two open-field tests following treatment with an NMDA blocker or vehicle solution.

AP5 animals received acute drug injections rather than minipump administration. In addition, all groups were tested in an open-field apparatus prior to milk maze training. This provided an explicitly novel spatial experience prior to milk maze training and might have functioned as a promoter of NMDA channel activation in the hippocampus. Finally, control animals which had acquired the milk maze problem were tested for retention in the presence of the NMDA blocking drugs. Testing control animals under NMDA blockade assesses the effects of blockade on retention and provides a control for any drug effects which might interfere with performance in the Morris milk maze. Theoretically, these drugs should not interfere with retention of previously acquired spatial memories.

METHOD

Subjects

Twenty-four male Long Evans rats obtained from Charles River Laboratories (Montreal, Quebec) at 150 g were used as subjects. The animals were individually housed in plastic cages with ad lib access to food and water in a vivarium maintained at 22°C with a 9-hr on/15-hr off light cycle. At the beginning of behavioral testing animals weighed 305–340 g.

Surgery

Twelve of the subjects underwent surgical implantation of a Plastic Products 22-gauge outer guide cannula containing an inner stylet which projected 0.5 mm beyond the guide cannula tip. The cannula assembly was directed at the right lateral ventricle. The stereotaxic surgeries were conducted using aseptic technique under sodium pentobarbital anesthesia (65 mg/kg IP). Prior to anesthesia each animal was given IP injections of atropine (0.2 mg) and acepromazine maleate (2 mg). Each cannula assembly was placed 0.9 mm posterior and 1.4 mm lateral to bregma with the head oriented in a skull flat position. The ventral placement was 2.4 mm ventral to the cortical surface. Following placement of the cannula, dental cement was used to anchor the cannula assembly to four skull screws. Surgeries were completed over a two-day period. All animals had at least ten days to recover from surgery prior to behavioral testing.

Apparatus

Open-field testing was carried out in a circular open-field maze 83.8 cm in diameter with white walls 32 cm high. The open-field wall was placed on a white formica table top which served as the floor.

The milk maze consisted of a white plastic circular liner, diameter 134.6 cm and wall height 51 cm, inserted into a child's wading pool. Powdered Carnation milk rendered the water opaque. A Plexiglas platform, $20.1 \times 15.2 \times 19.3$ cm, was placed in the northwest quadrant just below the surface of the milk which was at a depth of 20.8 cm. Both mazes were located in a large experimental room which was kept at approximately 23 degrees Celsius.

Drug Treatment

Experimental subjects were assigned to one of four treatment groups. Six animals received MK-801 IP (0.07 mg/kg in a 0.1 mg/ml volume) while six other animals were given 0.9% saline IP in injections of equal volume. The subjects with cannula implants received ICV injections of 10 μg AP5 (5 μg/μl, N = 6) or saline of equal volume (2 μl, N = 6). The 28-gauge injection cannula extended 1 mm below the tip of the outer guide cannula. The ICV injections occurred over a 90-sec period and the inner cannula was left in place for a further 90 sec.

Procedure

Subjects were run in groups of 4 with one member from each drug treatment condition (MK-801, MK-801 control, AP5, AP5 control) in each group. All subjects (N = 24) were run each day.

Days 1 and 2. Twenty minutes after IP injections, for MK-801 subjects, and ten minutes after ICV injections, for AP5 subjects, each animal in a group of 4 was placed individually in the center of the circular open field. Locomotor patterns were traced on scaled drawings (scale 1:5.5; 2.54 cm = 13.97 cm) of the open field for each minute of a three-minute open-field test.

Following the open-field test, and an approximately 10-minute period in the home cage, each of the four animals was given a training trial in the Morris milk maze. Animals were started from a predetermined location on the edge of the maze and allowed 54 seconds to find the platform. If subjects located the platform within 54 seconds they were given 10 seconds to remain on the platform before removal from the maze. If subjects failed to locate the platform in 54 seconds they were placed on the platform manually and left for 10 seconds before removal to the home cage. Each group of four animals was run in this manner for a total of six trials. The starting locations used were north, east, south and west. These four locations were randomly varied for each of the six trials. Behavior in the milk maze was video recorded and a stop watch was used to time latency to reach the platform.

At the conclusion of Days 1 and 2 each animal had been tested twice in the open field and received 12 acquisition trials in the Morris milk maze.

Day 4. Forty-eight hours after the two training days all animals were tested for their memory of platform location in the milk maze. No drugs were administered. The platform was removed from the maze. Each subject was started in the maze from the east side and observed in the maze for 60 seconds. The latency to reach the original platform location and the number of times each animal crossed the location were recorded.

Day 6. Forty-eight hours after the first retention test, a second test was run using only those 12 subjects who had been in the control conditions for each drug treatment. Three of the MK-801 controls were assigned to receive MK-801 injections 20 minutes prior to the retention test, while the remaining three received saline injections. Three of the AP5 controls received AP5 10 minutes

TABLE 1

MEAN CENTIMETERS TRAVELLED IN THE OPEN FIELD OVER DAYS

	Drug		Control	
	MK-801	AP5	MK-801	AP5
Day 1	760.9	826.6	400.8	538.5*
	(59.7)	(64.8)	(43.4)	(37.0)
Day 2	**1000.9**	**1111.8†**	345.2	477.6*
	(58.3)	(93.2)	(45.8)	(48.0)

Underlined pairs of means do not differ. Day 1 and Day 2 Controls differ from Day 1 and Day 2 Drug conditions (*$p<0.01$). Day 2 Drug means (bold) differ from Day 1 Drug means (†$p<0.05$). Standard errors of the mean are in parentheses.

TABLE 2

MEAN CENTIMETERS TRAVELLED IN THE OPEN FIELD OVER MINUTES

	Drug		Control	
	MK-801	AP5	MK-801	AP5
Min 1	884.3	957.5	434.8	**660.3*†**
	(72.0)	(128.4)	(61.4)	(51.9)
Min 2	875.7	989.1	382.9	456.9*
	(88.0)	(98.0)	(50.5)	(35.5)
Min 3	882.8	961.1	301.3	407.0*
	(84.4)	(96.1)	(47.9)	(39.1)

Underlined pairs of means do not differ. Drug means are significantly higher than Control means for each minute (*$p<0.01$). The AP5 Control mean (bold) is higher than the MK-801 Control mean in minute 1 (†$p<0.05$). Standard errors of the mean appear in parentheses.

prior to the retention test while three received saline. The second test was run in the same manner as the first.

Data Analysis

Cannula placements were examined at the conclusion of the experiment by injecting 10 μl of 0.5% thionin dye into the cannula after giving an anesthetic overdose of Euthanyl. Five minutes after dye injection animals were decapitated, the brains removed and hemisected sagittally to observe spread of the dye in the ventricle. Dye distribution was rated as poor, moderate or extensive.

Open-field activity was analyzed with the aid of Sigma-Scan. Each tracing was scanned and the distances travelled by each subject for each minute of the open-field test were calculated. Differences in distance travelled were analyzed with a three-way repeated measures ANOVA (drug treatment × day × minute; $4\times2\times3$).

Latencies to reach the platform during milk maze acquisition were also analyzed. If animals did not reach the platform in 54 seconds they were given a score of 54. A three-factor repeated measures ANOVA (drug × day × trial; $4\times2\times6$) was used to assess differences. For both the open-field and milk maze data Newman-Keuls tests were used to identify the origin of any significant differences.

For the two retention tests two-way ANOVAs were used to evaluate latency and crossing differences. *t*-Tests were used to assess the origin of group differences.

RESULTS

Open Field

Mean scores for the open-field data are displayed in Fig. 1 and in Tables 1 and 2. All two-way interactions were significant, drug treatments by days, $F(3,30)=3.9$, $p<0.024$ (see Table 1), drug treatments by minutes, $F(6,40)=2.8$, $p<0.02$ (see Table 2) and days by minutes, $F(2,40)=6.9$, $p<0.003$.

Animals given either a competitive (AP5) or a noncompetitive (MK-801) NMDA blocker were significantly more active than their respective controls on both Days 1 and 2 at each minute of the three-minute open-field test. There was a further significant increase in activity after NMDA blockade on Day 2. Control animals decreased in activity from Day 1 to Day 2, but the decrease was not significant. However, 11/12 experimental animals were more active at the end of the open-field testing than at the beginning (minute 1, Day 1 versus minute 3, Day 2) while 11/12 control animals were less active at the end of open field testing than they were at the beginning ($\chi^2=8.3$, $p<0.01$).

The days × minutes interaction reflected a difference in habituation over minutes on the two days (see Fig. 1). No significant decrease in minute to minute activity was seen overall on day 1 (mean day 1 = 631.7 cm), while on day 2 activity began at a higher level than day 1 (mean day 2, minute 1 = 837.8 cm) and decreased significantly over min 2 (mean = 724.9 cm) and minute 3 (mean = 639 cm).

Milk Maze Acquisition

In an overall analysis of escape latencies over the six training trials on days 1 and 2, there were three significant main effects: trials, days and groups (see Fig. 2). Over trials there was a significant decline ($p<0.01$) in escape latency from trial 1 (mean = 36.7 sec) to each of trials 2 through 6 (overall mean of trials 2–6 = 25.3 sec). Over days, escape latencies decreased significantly ($p<0.001$) from day 1 (mean = 33.7 sec) to day 2 (mean = 20.8 sec).

The drug treatment groups had significantly longer latencies than the control groups. The mean escape latencies of the MK-801 group (mean = 37.4 sec) and of the AP5 animals (mean = 33.5 sec) were significantly slower ($p<0.01$) than that of their respective control groups (mean of MK-801 control = 14.5 sec; mean of AP5 control = 23.2 sec).

The mean escape latencies of individual animals on Day 1 in the drug conditions (AP5 and MK-801 combined) correlated positively ($r=.77$, $p<0.003$), with the total distance travelled in the open field on the same day. Animals that were more active in the open field took longer to find the platform. Control group animals showed no similar correlation between open field and maze acquisition measures. The relationship was not significant for the drug groups on Day 2.

Milk Maze Retention Tests

The mean times to reach the platform location on the no drug retention test (see Table 3 and TEST in Fig. 2) 48 hours after the last training day were again significantly different, $F(3,20)=13.59$, $p<0.01$. The mean latencies of the MK-801 and AP5 groups were 45.8 and 40 seconds, respectively, while the MK-801

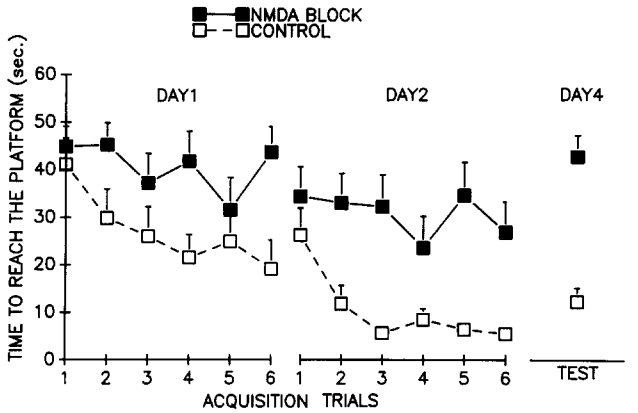

FIG. 2. Mean time to reach the platform on each of six acquisition trials following drug treatment on Days 1 and 2. Mean time to reach the platform area is also shown for the same groups on the retention test without drug administration on Day 4. Vertical bars indicate standard errors of the means.

control group and the AP5 control group reached the platform location in 12 and 12.7 seconds, respectively, $t(10)$MK801 vs. control $= 3.92$, $p<0.01$; $t(10)$AP5 vs. control $= 3.2$, $p<0.01$]. The two control groups did not differ from each other, nor did the two drug groups.

Subject crossings over the platform area were also significantly different. MK-801 animals crossed the target area 1.18 times, while MK-801 controls crossed the area 3.38 times, $t(10) = 2.36$, $p<0.05$. AP5 animals crossed 0.83 times, while their controls crossed the area 3.67 times, $t(10) = 3.32$, $p<0.01$.

Retention Test Under the Drug

In the second retention test on Day 6 only the behavior of the control animals was examined, but half of each control group was given the drug treatment and tested under the drug. There were no effects of the drug treatment on this retention test (see Table 3). The mean latency of the MK-801 treated animals was 7.3 seconds to reach the platform location, while MK-801 control animals took 19.7 seconds. The mean latency of the AP5-treated animals was 11.3 seconds, while AP5 controls took 25 seconds. Mean crossings also were not different with 2.67 crossings for the MK-801 drugged subjects and 4.33 crossings for the AP5 subjects, both control groups had mean crossings of 3.33.

Histology

Among the 12 ICV implants, dye injection spread was moderate to extensive in the ventricle in eleven subjects. One subject from the AP5 group had poor dye distribution in the ventricle, but the data of this subject were not significantly different from the remainder of that group.

TABLE 3

MEAN LATENCIES TO REACH THE PLATFORM AREA AND MEAN CROSSINGS IN THE PLATFORM AREA ON TWO TESTS OF RETENTION

	NMDA Block			Control		
	n	Latency	Crossings	n	Latency	Crossings
Drug During Acquisition	12	42.9 sec (4.5)	1.0 (0.3)	12	12.3 sec (2.9)	3.8 (0.6)
Drug During Retention	6	9.3 sec (1.2)	3.5 (0.7)	6	22.3 sec (9.8)	3.3 (0.8)

Standard errors of the mean appear in parentheses.

DISCUSSION

Both AP5 and MK-801, in bolus injections, increased activity in the open field and prevented acquisition of the milk maze problem. The pattern of effects with the two blockers was remarkably similar. When tested without blockers, after 12 training trials in the presence of blockers, the experimental groups were significantly worse in swimming to the area of the platform and in remaining in the area of the platform. Control animals, given the same amount of training and then tested under drug conditions, showed excellent retention of platform location, swimming directly to the platform location and making multiple crossings of the platform area (Table 3). NMDA blockade by either a competitive or noncompetitive blocker had a selective effect on acquisition, but not retention, of the Morris milk maze.

The increased open-field activity is consistent with a hypothesis of hippocampal dysfunction. Hippocampally damaged animals show increased open-field activity. O'Keefe and Nadel (10) hypothesize that this increase is attributable to reactivity to successively encountered stimuli and that, in the absence of spatial encoding, no normal exploration behavior occurs. In the present study no attempt was made to evaluate the quality of open-field activity, however, the strong correlation between open-field activity and escape latency impairment after NMDA block on Day 1 suggests a common mechanism may be reflected in the two tasks.

On the other hand, the significant increase in open-field activity on the second day of drug administration is less clearly related to the hypothesized hippocampal dysfunction. Why would reactivity increase over days? This increase could reflect sensitization to, or cumulation of, drug effects. Activity levels on Day 2 did not correlate significantly with escape latencies. NMDA blockers may increase activity by a mechanism which is independent of their interference with spatial coding.

Whether or not the significant increases in activity reflect solely a loss of spatial encoding, it is clear that animals can successfully and efficiently perform the milk maze task under the influence of an NMDA blocker when acquisition of the map has occurred prior to drug administration.

MK-801 effects were similar to AP5 effects in both the open field and the milk maze. It appears that, in these animals, there was sufficient activity of the NMDA channels to produce effective blockade by MK-801 within 20 minutes of drug administration. Halliwell and Morris (3) also waited 20 minutes before testing and used a 0.1 mg/kg dose of MK-801. In pilot work we found 0.03 mg/kg was effective in Sprague-Dawley animals, while 0.07 mg/kg was necessary with Long-Evans rats. Higher doses increased the probability of ataxia, but did not appear to alter the effect on acquisition. The threshold for effective doses appears critical since lower doses gave no observable effects in the pilot experiments. Nonetheless, the present doses were lower than those used by Halliwell and Morris. Dose discrepancy does not offer an explanation of the difference in results in the two experiments.

The present data support the hypothesis that the NMDA receptor is critically involved in the acquisition, but not the retention, of spatial information. This is consistent with the hypothesis that NMDA receptors are involved in the initial encoding of spatial information but not in its maintenance. However, recent data from Sutherland (12) indicate that retention in the Morris milk maze can survive general hippocampal dysfunction. Retention is successful if the interval between training and damage is sufficiently long (12 weeks). It would seem

unlikely that NMDA blockers are producing a hippocampal lesion-like dysfunction, since retention under the blockers can be exhibited within a week of original acquisition. However, we do not know when or why spatial information transfer occurs and it may be that the surgical trauma of traditional lesion approaches contributes to the long interval required for demonstration of transfer. The question of locus of spatial information storage is critical to the analysis of drug effects on acquisition and retention.

The hypothesized role of NMDA receptors in synaptic plasticity and learning is attractive. The present data are consistent with that hypothesis but, given Sutherland's results, they do not rule out the possibility that the NMDA blockers produce a general hippocampal dysfunction [see also (6)]. A stronger case for robust hippocampal encoding after NMDA blockade would depend on unchanged place cell firing in a familiar environment.

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