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A Novel NMDA Antagonist, MK-801, Impairs Performance in a Hippocampal-Dependent Spatial Learning Task

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BUTELMAN, E R *A novel NMDA antagonist, MK-801, impairs performance in a hippocampal-dependent spatial learning task* PHARMACOL BIOCHEM BEHAV **34**(1) 13–16, 1989 —N-Methyl-d-aspartate (NMDA) receptors have been implicated with the triggering of long-term potentiation, a currently studied physiological model of learning and memory The compound (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate (MK-801) has recently been classified as a potent and selective NMDA antagonist acting at the associated ion channel After determination of the highest intraperitoneal dose of MK-801 at which increases in activity (measured in photocell activity cages and 3-arm maze) were not observed (0 2 mg/kg), rats that had been previously trained to obtain food pellets in an 8-arm radial maze up to criterion were tested with 0 1 and 0 2 mg/kg doses Dose-related decreases in "efficiency" in the task were found The present findings support the suggestion that NMDA antagonists cause impairments in "working memory" and also support the status of long-term potentiation as a physiological model of memory

NMDA receptor MK-801 Long-term potentiation Radial arm maze Working memory

THE N-methyl-d-aspartate (NMDA) subtype excitatory amino acid (EAA) receptor has been found to mediate the triggering, but not the maintenance of the process of long-term potentiation (LTP), a current physiological model of learning and memory (2, 8, 16, 20) NMDA receptor function is also significant during high frequency presynaptic firing and in magnesium-free medium (4,11) The highest concentrations of NMDA receptors in the rat brain are in the hippocampus which is also the main site of observation of LTP (7,20)

Chronic intracerebroventricular infusion of an NMDA receptor antagonist, (d)-2-amino-5-phosphonovalerate (APV), in the rat, impaired the ability to learn a spatial memory task (17), but did not produce observable behavioural changes at large doses when systematically administered (14). It has recently been found that intracerebroventricular injections of APV in the rat cause impairments in performance in radial arm maze (RAM) (9). The dissociative anesthetic phencyclidine (PCP), which has important NMDA antagonist properties (1) and blocks LTP in vivo (23), also affects performance in the RAM (9,15)

The compound MK-801 has recently been found to have potent and selective noncompetitive NMDA antagonist properties (10,25) Recently, it has also been investigated for its potent "antineurodegenerative" and anticonvulsant properties (13,24), which seem to result from its efficiency as an NMDA antagonist MK-801 blocks LTP in the rat hippocampal slice, leading to the proposal that it could impair cognitive functions via this effect (5)

Hippocampal damage in the rat has been reported to result in a disruption of performance in the radial arm maze (RAM), a spatial "working memory" task (18) The purpose of the present investigation was to study the effect of intraperitoneally administered MK-801 in the radial arm maze in order to evaluate the proposal that NMDA neurotransmission (and LTP) are implicated in memory Dose levels at which no overt motor or stereotypical effects were observable were used (0.1, 0 2 mg/kg), in order to exclude their relevance to performance in the RAM Similar dose levels were found to antagonize NMDA-induced effects in the rat and gerbil (12,22) The RAM task was chosen because of its dependence on hippocampal function, which is sensitive to MK-801 administration.

METHOD

Subjects

Male Lister Hooded rats (Harlan Olac, Bicester, UK, weights 250–350 g at testing) were housed in pairs with a 12-hour light/dark cycle and ad lib access to food and water Testing in the activity cage and 3-arm experiments took place between 0900–1400. In the RAM experiment Ss were maintained at 85 percent of free-feeding weight throughout training and testing, which took place between 0900 and 1600

Apparatus

Activity cages Plexiglas activity cages were 46 × 23 × 20 cm

deep Two parallel photocell beams were placed across the cage at a height of 4 cm and 26 cm apart from each other Activity counts were obtained for consecutive 5-minute periods Throughout the 3 studies, the experimental room was dimly lit to allow behavioural observation

Three-arm maze A grey brick 3-arm maze with 22 cm high walls on a linoleum floor was used The arms were at 120 degrees orientation from each other (arm width 10 cm, arm length 42 cm)

Eight-arm maze The radial arm maze (19) was wholly constructed in unpainted aluminium and was elevated at a height of 50 cm, the arms were 75 cm long and 7 5 cm wide, slightly tapering at the end proximal to the central octagonal platform (side 14 cm) A food well was found at the end of each arm The room containing the maze had various visual cues (table, chairs, etc) Noyes food pellets (45 mg) were used

Procedure

Activity cages Subjects had 2 hours habituation in the cage, one day previous to drug testing On test days, 8 subjects were assigned to control or experimental conditions (N = 4 per condition) for each dose level (treatment), they were injected in quick succession and tested for activity for a continuous 1-hour period Control Ss received an equivalent volume injection of vehicle solution Behavioural observations were also carried out The drug MK-801 was dissolved in saline and administered in doses 0 1, 0 2, 0 3 and 0 4 mg/kg Activity data were compared across doses and conditions

Three-arm maze Eight Ss were divided between control and experimental conditions for each treatment (N = 4 per condition), control Ss receiving equivalent vehicle injections The treatments tested were 0 1, 0 2, 0 3 mg/kg MK-801 The frequency of arm entries was compared across conditions Arm entries were visually scored as the presence of the whole body (minus the tail) within an arm, in two 5-minute trials Testing started immediately after injection with an inter-trial interval (ITI) of 30 minutes The 3-arm maze activity data were used to complement and validate dose-activity counts obtained in activity cages (21)

Radial arm maze Fourteen Ss were trained in a standard RAM task, receiving 3 trials per day (ITI 60 minutes approximately), two days per week (Mondays and Thursdays or Tuesdays and Fridays), receiving overall 6 trials/week Before a trial was started, one food pellet was placed in the food well at the end of each arm, a perfect trial would therefore occur if the S retrieved all the pellets with 8 arm entries A trial lasted until a subject visited all the arms in the maze, up to a maximum of 10 minutes Arm entries were scored visually as the presence of 4 paws within an arm Competence in the task was monitored by the computation of an "efficiency" value for each trial [as defined in (15)] being the ratio 8/(total number of arms entered in the trial) expressed as a percentage For example, in a trial where 10 arm entries were made before the 8 arms had been entered, the efficiency value would be 8/10 or 80 percent Training continued to criterion, which was defined as 3 consecutive trials at 80 percent efficiency or above

Two treatments were studied for each subject 0 1 and 0 2 mg/kg MK-801 After criterion, subjects were tested in both conditions in random order, tests being separated by at least 2–3 days On a test day, the S would receive 1 control and 1 experimental trial (separated by 4–5 hours) The condition order within test days was balanced across Ss Performance in experimental trials was compared within Ss with that of the control trial of the same day, which would be preceded by an equivalent vehicle injection The time taken to visit the first 8 arms entered in a trial was used to monitor activity levels in the task

TABLE 1

EFFECT OF VARIOUS INTRAPERITONEAL MK-801 DOSES ON ACTIVITY COUNTS

Dose MK-801 (mg/kg)	Experimental Mean (SEM)	Control Mean (SEM)
0 1	27 5 (4)	25 4 (4 7)
0 2	25 6 (4 9)	30 2 (6 1)
0 3	59 6 (12 8)	28 0 (2 1)
0 4	71 9 (5 6)	22 6 (3 5)

Mean photocell count of 12, 5-minute periods in the first hour postinjection N = 4 for each group

Drugs

The compound used was (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate (MK-801, Merck, Sharp and Dohme, Harlow, UK) at 0 2 mg/ml in saline (pH = 6), control subjects received vehicle solutions of the appropriate volume for each treatment Fresh drug solutions were prepared on each test day

RESULTS

Data obtained from the activity cage and 3-arm maze paradigms showed that MK-801 caused dose-related stereotypy, ataxia and increases in activity in the first hour postinjection The threshold dose for these effects was 0 3 mg/kg MK-801, and at this dose and above, a clear behavioural profile could be observed The two subthreshold doses (at which the activity level was unaffected) tested in the RAM, were found to cause dose-dependent decreases in efficiency The 0 05 significance level was adopted throughout for rejection of null hypotheses

Activity Cages

Table 1 illustrates the mean activity counts for the first hour postinjection The statistical analysis was carried out with a single two-way ANOVA There were significant dose and condition main effects as well as significant interaction between the two independent variables [condition main effect $F(1,24) = 19\ 54$, dose main effect $F(3,24) = 5\ 82$, interaction $F(3,24) = 8\ 13$]

A Newman-Keuls test subsequently revealed that the 0 3 and 0 4 mg/kg MK-801 conditions had significantly higher activity counts than all others, but did not differ among themselves The 0 1 and 0 2 mg/kg MK-801 conditions did not differ from any control value or from each other Behavioural observations indicated the presence of ataxia, head sways and increased locomotion (the latter detected as an increase in activity count) at the 0 3 and 0 4 mg/kg doses, while no stereotypical effects were seen at the 0 1 and 0 2 mg/kg doses

Three-Arm Maze

Three separate *t*-tests were computed between control and experimental Ss at each dose level of MK-801 in the 3-arm maze The mean number of arm entries (as an index for activity levels) was significantly increased in the 0 3 mg/kg MK-801 condition

TABLE 2

EFFECT OF MK-801 ON ACTIVITY IN THE 3-ARM MAZE

Dose Level (mg/kg)	Experimental Mean (SEM)	Control Mean (SEM)
0 1	19 3 (2 3)	17 3 (1 9)
0 2	13 5 (3 4)	13 0 (1 2)
0 3	23 6 (3 4)	14 6 (3 1)

Mean number of arm entries in two 5-minute periods, first hour postinjection N = 4 per group

only, $t(6) = 1\ 95$, thus confirming the findings in the activity cage experiment, indicating the latter dose as the threshold for activity increases in the first hour postinjection Values are provided in Table 2

Radial Arm Maze

All Ss except one (which was excluded from the study due to failure to reach criterion) readily learnt the RAM task The mean number of trials to criterion was 16 (SEM = 1 1, range = 10 to 23), N = 13

The 0 1 and 0 2 mg/kg MK-801 doses were found to impair dose-dependently the Ss' ability to carry out the RAM task A two-way ANOVA revealed a significant condition effect, $F(1,12) = 22\ 5$, while a Newman-Keuls test showed that efficiency values under both experimental doses were significantly lower than those found in control trials (the two control doses did not significantly differ from each other) (see Table 3) However, a Wilcoxon test, $T(13) = 21$, on (experimental/control) ratios showed that the efficiency levels differed significantly between the experimental doses, relative to control levels (0 1 mg/kg MK-801 ratio mean = 89 9, SEM = 6 8, 0 2 mg/kg MK-801 ratio mean = 74 4, SEM = 5 5) This would suggest that efficiency was impaired in a dose-dependent manner

Activity levels (as measured by the time taken to enter the first 8 arms in a trial) were not affected by either of the MK-801 doses used in the RAM study (data in Table 4), confirming the findings from the activity cage and 3-arm maze experiments

The possibility that a "carry-over" effect could affect control performance on test days in which saline injections were preceded by MK-801 injections, was investigated Efficiency levels were compared between control trials occurring before the experimental trial ("preceding") or after it ("following") on each test day The efficiency data were analysed for the two MK-801 doses with Mann-Whitney tests, and no significant differences were found

TABLE 3

EFFICIENCY LEVEL IN RAM WITH VARIOUS MK-801 DOSES

Dose MK-801	Experimental Mean% (SEM)	Control Mean% (SEM)
0 1 mg/kg	74 (4 6)	84 (3 1)
0 2 mg/kg	62 (4 8)	84 (4 1)

Efficiency = (8/total number of arm entries) expressed as a percentage N = 13

TABLE 4

ACTIVITY IN THE RAM WITH VARIOUS MK-801 DOSES

Dose MK-801	Experimental Mean (SEM)	Control Mean (SEM)
0 1 mg/kg	72 (4 5)	72 (4 1)
0 2 mg/kg	70 (5 4)	66 (5 5)

Time(s) to the first 8 arm entries in a trial N = 13

(data in Table 5) This finding would indicate that control trials given before or after experimental trials did not significantly differ from each other with respect to efficiency, and that it is unlikely that the control level of efficiency was affected by experimental injections earlier in a test day Therefore, the efficiency impairment caused by MK-801 became unobservable from 4 hours postinjection

DISCUSSION

The aim of this investigation was to study the effects of MK-801 on hippocampal-dependent performance in the RAM The results indicated that 0 1 and 0 2 mg/kg doses caused impairments in efficiency in the RAM task, in the absence of motor or stereotypical effects The possibility of a "carry-over" effect on control trials following experimental trials within a test day was considered It was found that control trials occurring before or after experimental trials within a test day did not differ from each other with respect to efficiency

The impairment in efficiency in the RAM under the effect of MK-801 may be interpreted as an NMDA-mediated disruption of working memory Two lines of evidence suggest that the compound caused the above effect by acting on the hippocampus firstly, the fact that hippocampal-lesioned rats show very poor performance in the RAM task (18), secondly, the occurrence of NMDA receptors in the rat is especially high in the hippocampus (20) The validity of LTP as a physiological model of memory is also supported by these findings, due to the fact that the induction of LTP in the hippocampus is blocked by MK-801 (5)

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TABLE 5

CONTROL EFFICIENCY LEVEL IN THE RAM PRECEDING OR FOLLOWING EXPERIMENTAL TRIALS

Dose MK-801	"Following" Mean (SEM)	"Preceding" Mean (SEM)
0 1 mg/kg	84 6 (4)	82 6 (5 4)
0 2 mg/kg	80 8 (6 2)	86 4 (5 6)

"Following" control trials occurred after experimental trials in the same day (the opposite is true for "Preceding" control trials)

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