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Effects of Ketamine and l-Glutamic Acid Diethyl Ester on Concept Learning in Rats

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LALONDE, R. AND C. C. JOYAL. *Effects of ketamine and l-glutamic acid diethyl ester on concept learning in rats.* PHARMACOL BIOCHEM BEHAV **39**(4) 829–833, 1991.—The effects of ketamine, an NMDA receptor antagonist, and l-glutamic acid diethyl ester (LGDE), a non-NMDA glutamate antagonist, were evaluated in the acquisition of concept learning in a water maze. In concept learning, the rats must locate an invisible platform whose location changes from day to day. In spatial learning (Morris task), the rats must locate an invisible (or visible) platform whose location does not change. Ketamine increased quadrant entries at 5, 10 and 20 mg/kg, and latencies at 10 and 20 mg/kg on the final two days of training on the concept task. At 5 mg/kg ketamine disrupted concept learning but not spatial learning or visuo-motor coordination as assessed by invisible and visible platform conditions of the Morris maze. Progressively higher doses of ketamine affected first the invisible condition and then the visible platform condition. On the other hand, LGDE did not affect the Morris task at any dose. However, there was no decrease in latencies over days in concept learning at the two highest doses (240 and 360 mg/kg) of LGDE. Thus LGDE appeared to slow down decision time in the concept task but not the spatial task in the absence of an effect on quadrant entries in any version. These results indicate that NMDA receptors are involved in spatial and concept learning. Non-NMDA receptors appear to be involved only in concept learning.

Ketamine Concept learning Water maze L-Glutamic acid diethyl ester NMDA receptor

KETAMINE is a dissociative anesthetic with limited use in humans because of unacceptable side effects such as confusion, agitation and hallucinations (4). There has been renewed interest for this drug due to its noncompetitive NMDA antagonist properties (18). Ketamine, like other NMDA antagonists, prevents neuronal degeneration caused by ischemia and hypoxia in animals (12, 15, 20). At higher concentrations, ketamine prevents anoxic injury to white matter as assessed in the rat optic nerve model (14). It has been suggested that the protective effects of ketamine on white matter are related to its anesthetic effects on ion permeability and cellular membrane potential (14). Because NMDA antagonists are known to impair learning ability (1–3, 7, 9, 13, 17), it is of interest to study further the behavioral effects of ketamine.

For this purpose, concept learning in a water maze was used. In one version of the Morris test, an animal is placed in various orientations with the platform in the same position, and the animal must guide its turns towards the platform by using extra-maze visual cues (13). A second version of this test requires concept learning, in that the position of the platform is changed from day to day instead of remaining stable (21). This version requires concept learning in that although the spatial position of the platform changes from day to day, the animal is still able to reach it more quickly. Thus the animal is said to have acquired the ability to learn how to learn or a learning set (21). It is assumed that the animal learns such procedural skills as avoiding to explore the sides of the basin because no escape is available there. The animal learns that the only way to escape the water is by localizing the position of the platform.

Acquisition of the Morris task is impaired by NMDA antagonists (1, 9, 13), but the effects of these drugs on concept learning have not been evaluated. The non-NMDA glutamate antagonist, l-glutamic acid diethyl ester (LGDE) (5,19), was also assessed. The administration of non-NMDA glutamate antagonists can also prevent neuronal injury (16) and causes learning deficits (7, 8, 10). LGDE was assessed at dosages between 120 and 360 mg/kg 30 min after injection mainly because significant learning deficits were found in bar-pressing and discrimination learning tasks (8,10) at these dosage levels and this postinjection interval. Ketamine was assessed at 5–20 mg/kg 20 min postinjection on the basis of learning deficits recorded with these approximate parameters (1).

The effects of the two drugs were also evaluated in the version with a stable platform in either visible or invisible platform conditions (13). Two measures were used: the number of quadrant entries and latencies until reaching the platform. The pool being separated into four quadrants, the number of quadrant entries is a measure of the path taken to reach the goal, a high number meaning that the path was more indirect (13).

METHOD

Subjects

Four series of 28 male Sprague-Dawley rats with a mean

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starting weight of 350–450 g were used (Charles River Canada, St-Constant, Quebec). The rats were kept in a temperature and humidity-controlled room with a 12-h light-dark cycle (lights off at 1830). Food and water were available at all times.

Apparatus

A rectangular water basin made of metal and measuring 108 × 66 cm (height: 51 cm) was used. The depth of the water was 24.5 cm and the temperature of the water about 28°C. The circular platform (diameter: 4.5 cm), an inverted glass beaker covered with wire mesh to provide firm gripping, was placed 5 cm below water level.

Procedure

In the first experiment, the 28 rats were separated into 4 groups according to the dose levels of ketamine (Ketalar, Parke-Davis, Montreal): 0, 5, 10 or 20 mg/kg IP, 20 min before testing diluted in 0.9% saline (injection volume = 1 ml/kg for the 0, 5 and 10 mg/kg doses and 2.0 ml/kg for the 20 mg/kg dose, calculated form the base). On the first day, as well as on each subsequent day, the rats were placed in the water facing the wall in the north (N) position, followed by the east (E), south (S) and west (W) positions in that order. There were 8 trials per day, with an intertrial interval of 10–15 s (spent in a plastic container outside the maze) for trials 1–4 and 5–8 and 2–4 min (spent in their home cage) between trial 4 and trial 5. The cut-off point in the maze was 60 s, and, on reaching the platform, the rat was allowed to stay on it for 5 s. Time was determined by a stopwatch and quadrant entries by means of visual cues (white tape) in the maze. The experimenter was present in the room, richly furnished with visual cues such as a window, cupboards and surgical instruments, and remained in the same position (S). The position of the invisible platform was NW on day 1 and then changed in a clockwise orientation for the next three days.

In the second experiment, the same procedure was followed, except that LGDE (Sigma, St. Louis, MO) was injected IP 30 min before the learning session at one of four dose levels = 0, 120, 240 and 360 mg/kg (dissolved in 0.9% saline, injection volume = 1 ml/kg).

In the third and fourth experiments, the same general procedure was followed, except that a stable platform was used in either invisible or visible platform conditions (spatial learning). During the first 3 days, the platform, situated in the NW position, was invisible. Thus the platform remained stable from day to day over 3 days and acquisition of this task was assessed. The platform was moved only on days 4–5 and placed in the SE position. This assessed acquisition of a spatial task after original learning (reversal condition). On day 6, the platform was elevated above water level by means of metal plates at the bottom of the pool and placed in the SW position. This assessed visuomotor coordination towards a visible platform. LGDE was tested at the same dose range and postinjection interval as above, while ketamine was tested at 5–15 mg/kg 20 min postinjection.

Statistical Analyses

In concept learning, mean values of the tables represent total quadrant entries and latencies summed over the first 16 trials (days 1 and 2 combined) and the last 16 trials (days 3 and 4 combined). In all experiments, homogeneity of variances was evaluated by means of the Greenhouse-Geisser test (6). In cases of homogeneous variances, parametric tests such as ANOVA and paired *t*-tests were used. In cases of heterogeneous variances, overall group comparisons were made by means of the Kruskal-Wallis test, and individual group comparisons were made by the Wilcoxon rank sum or the Wilcoxon matched-pairs signed ranks tests. The Wilcoxon rank sum test was used as a multiple comparison test, with a correction for significance set at 0.05/3 (because of 3 comparisons of interest) = 0.01 (11).

TABLE 1

MEAN (SD) QUADRANT ENTRIES OF RATS INJECTED WITH KETAMINE OR L-GLUTAMIC ACID DIETHYL ESTER

	Days 1–2	Days 3–4
Ketamine Dose (mg/kg)		
0	80.9 (12.8)	44.9 (6.1)
5	79.1 (28.9)	57.9 (11.0)*
10	76.1 (20.5)	62.0 (18.8)*
20	89.1 (19.4)	80.4 (19.7)†
LGDE Dose (mg/kg)		
0	63.0 (11.5)	45.0 (2.8)
120	66.3 (4.1)	47.3 (6.7)
240	75.1 (15.4)	57.0 (15.9)
360	71.0 (13.3)	50.6 (12.6)

*$p<0.05$ vs. 0 mg/kg (Newman-Keuls multiple comparison).
†$p<0.01$ vs. 0 mg/kg (Newman-Keuls multiple comparison).

RESULTS

Concept Learning

There were 4 dosages of ketamine and 2 time periods (days 1–2 combined vs. days 3–4 combined). For quadrant entries, a 4 × 2 ANOVA with repeated measurements on the days factor revealed a significant days effect, $F(1,24) = 38.5$, $p<0.01$, and a significant interaction, $F(3,24) = 3.35$, $p<0.05$, but not a significant dose effect, $F(3,24) = 1.96$, $p>0.05$. As shown in Table 1, the days effect is due to a drop in the number of quadrant entries over days. The significant interaction term is explained by the fact that although all four groups had lower quadrant entries over days, the drop was less steep in the case of the ketamine groups. Quadrant entries on days 3–4 were higher in comparison to placebo for ketamine doses 5, 10 mg/kg, both ($p<0.05$) and 20 mg/kg ($p<0.01$) Newman-Keuls multiple comparison test.

There was a drop in latencies over days at 0, 5 and 10 mg/kg of ketamine, $W_+(7) = 0$, $p<0.01$, in all cases, but not at 20 mg/kg, $W_+(7) = 6$, $p>0.05$. As shown in Table 2, the main effect of the drug was to increase latencies on days 3–4, $H(3) = 15.28$, $p<0.01$. Latencies were higher on days 3–4 in comparison to placebo for ketamine groups 10 mg/kg, $R_1(7,7) = 34$, $p = 0.01$, and 20 mg/kg, $R_1(7,7) = 28$, $p<0.01$.

A 4 × 2 ANOVA was determined for quadrant entries according to 4 doses of LGDE over days. There was a significant days factor, $F(1,24) = 39.98$, $p<0.01$, but not a significant dose factor, $F(3,24) = 2.44$, $p>0.05$, or interaction, $F(3,24) = 0.11$, $p>0.05$. All four groups had lower quadrant entries over days, $t(6) = 3.66$, $p<0.01$, for placebo; $t(6) = 4.87$, $p<0.01$, for 120 mg/kg; $t(6) = 2.59$, $p<0.05$, for 240 mg/kg; and $t(6) = 2.48$, $p<0.05$, for 360 mg/kg of LGDE.

Although no effects were detected for LGDE in terms of

TABLE 2

MEAN (SD) LATENCIES (S) OF RATS INJECTED WITH KETAMINE OR L-GLUTAMIC ACID DIETHYL ESTER

	Days 1–2	Days 3–4
Ketamine Dose (mg/kg)		
0	334.3 (90.6)	122.7 (16.5)
5	347.1 (128.3)	171.9 (35.5)
10	320.7 (138.5)	216.3 (94.3)*
20	386.4 (147.5)	275.4 (83.8)*
LGDE Dose (mg/kg)		
0	226.6 (88.4)	117.1 (17.9)
120	242.0 (38.8)	147.7 (41.8)
240	306.6 (55.8)	229.3 (160.4)
360	324.4 (120.2)	299.4 (273.8)

*$p \leq 0.01$ vs. 0 mg/kg (Wilcoxon rank sum).

quadrant entries, this was not so in terms of latencies (Table 2). There was a significant drop in latencies for the placebo group, $W_+(7)=1$, $p<0.01$, and at 120 mg/kg, $W_+(7)=0$, $p<0.01$, but not at 240, $W_+(7)=7$, $p>0.05$, or 360 mg/kg, $W_+(7)=10$, $p>0.05$, of LGDE. Although latencies were higher on days 3–4 at higher doses of LGDE, this result did not reach significance, perhaps due in part to extremely high variability of responses, $H(3)=6.18$, $p>0.05$.

Spatial Learning

During days 1–3, significant main effects of trials, $F(1,24)=40.38$, $p<0.01$, and dosages, $F(3,24)=9.76$, $p<0.01$, occurred for quadrant entries. A significant main effect of dosages, $H=15.35$, $p<0.01$, also occurred for latencies. There was a drop in quadrant entries on trials 13–24 in comparison to trials 1–12 among ketamine group 0, $t(6)=9.11$, $p<0.01$, 5, $t(6)=5.92$, $p<0.01$, and 10, $t(6)=5.14$, $p<0.01$, but not 15 mg/kg, $t(6)=0.75$, $p>0.05$. The same pattern emerged in terms of latencies, with a drop ($p<0.01$) among ketamine groups 0, 5 and 10, but not 15 mg/kg ($p>0.05$) (Wilcoxon matched-pairs signed ranks test). Combined scores on days 1–3 indicated a higher number of quadrant entries in comparison to saline control for ketamine groups 10 ($p<0.05$) and 15 ($p<0.01$), but not 5 mg/kg ($p>0.05$) (Newman-Keuls multiple comparison test). The same pattern emerged for the latency measures, with higher latencies observed in comparison to saline control for ketamine groups 10 and 15 ($p<0.01$), but not 5 mg/kg ($p>0.05$) according to the Wilcoxon rank sum test as a multiple comparison procedure with correction for significance at $0.05/3=0.01$.

During days 4–5, the invisible platform was switched to a new position. ANOVA for quadrant entries revealed a significant main effect for dosages, $F(3,24)=5.25$, $p<0.01$. Newman-Keuls multiple comparison indicated a higher number of quadrant entries for ketamine group 15 ($p<0.01$), but not 5 or 10 mg/kg ($p>0.05$). Kruskal-Wallis analysis for latencies revealed a significant dosage effect, $H=11.42$, $p<0.01$. Higher latencies in comparison to the saline group were found for ketamine at 15, $R_1=44$, $p<0.05$, or 10 mg/kg, $R_1=40$, $p<0.05$.

On day 6, the platform became visible to all areas of the pool. Quadrant entries on that day gave heterogeneous variances, and so a $\sqrt{x-0.5}$ transformation of the data was performed, yielding homogeneous variances. Significant dosage effects emerged for both quadrant entries, $F(3,24)=8.16$, $p<0.01$, and latencies, $H=10.6$, $p<0.05$. Higher quadrant entries and latencies were found for ketamine at 15 ($p<0.01$), but not at 5 or 10 mg/kg ($p>0.05$) in comparison to saline. Figure 1A and B illustrates the dose-dependent impairment of ketamine on both visible and invisible platform conditions.

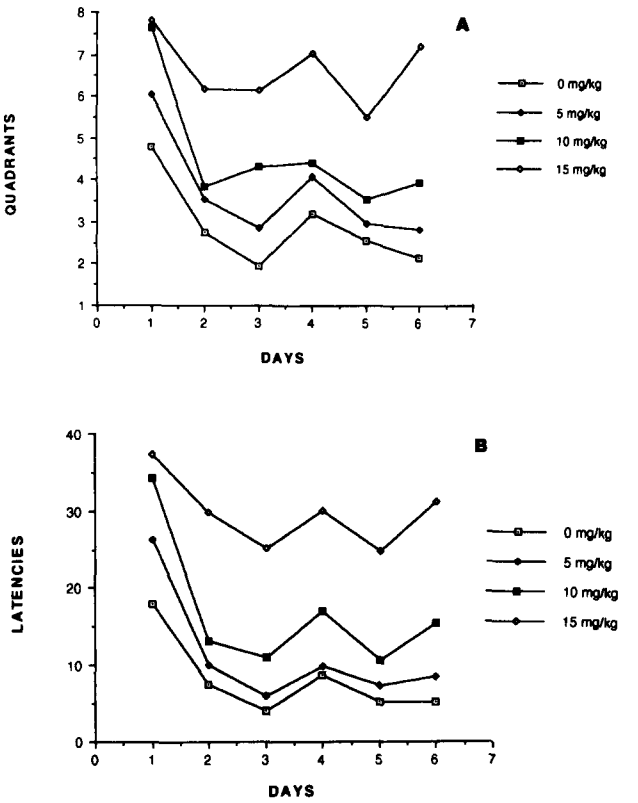

FIG. 1. Effects of ketamine on quadrant entries and latencies (s) in a spatial learning task in a water-maze. On days 1–3, rats learned to reach an invisible platform in the NW position. On days 4 and 5, the position of the invisible platform was changed to the SE position. On day 6, the platform was visible at the SW position.

The effects of LGDE were evaluated in the same task. As shown in Fig. 2A and B, there was no difference between LGDE groups 0, 120, 240 or 360 mg/kg in terms of quadrant entries and latencies on days 1–3, 4–5 or 6.

DISCUSSION

As shown previously by Whishaw (21), normal rats can learn to decrease their quadrant entries and their latencies in the concept learning version of the Morris test in which the location of the invisible platform changed from day to day. In the present experiments, there was a significant drop for both measures on the part of the control groups over a 4-day period, demonstrating rapid learning of the task. The main effect of ketamine was to increase quadrant entries and latencies on days 3–4.

Higher quadrant entries found at 5, 10 and 20 mg/kg, and higher latencies at 10 and 20 mg/kg, are especially noteworthy because of the absence of visuo-motor defects at these two doses as evaluated by performance on the visible platform condition (Fig. 1A and B). At 10 mg/kg of ketamine, there was a deficit

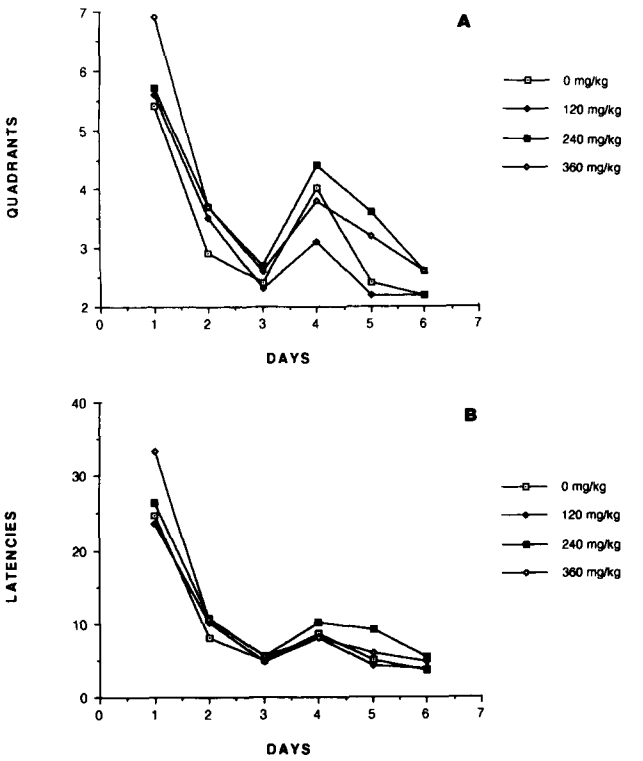

FIG. 2. Effects of l-glutamic acid diethyl ester on quadrant entries and latencies (s) in a spatial learning task in a water-maze. On days 1–3, rats learned to reach an invisible platform in the NW position. On days 4 and 5, the position of the invisible platform was changed to the SE position. On day 6, the platform was visible at the SW position.

in both acquisition of the spatial task (invisible stable platform) and the concept task. At 5 mg/kg of ketamine, there was a deficit only in the concept task, but not the spatial task. The latter deficit manifested itself as an increase in quadrant entries, latencies being higher but not significantly so (Fig. 1A and B). This is thus evidence of a gradation concerning the effects of ketamine according to dose levels. A high dose (15 mg/kg) disrupts visuo-motor coordination. A lower dose (10 mg/kg) disrupts spatial learning, but not visuo-motor coordination. An even lower dose (5 mg/kg) disrupts concept learning, but not spatial learning or visuo-motor coordination.

Contrary to ketamine, LGDE did not impair spatial learning (original or reversal conditions) or visuo-motor coordination at any dose level. The only effect found with LGDE was in the concept learning task. At 240 and 360 mg/kg of LGDE, there was no decrease in latencies over days (Fig. 2A and B). The drug slowed down the rats without making them swerve away from the goal as assessed by quadrant entries. A differential pattern of higher latencies in the absence of higher quadrant entries may simply mean that a drug reduces swimming speed. However, this effect was not observed in spatial learning or visuo-motor coordination. It is only in the concept learning task that an effect of LGDE on latencies was observed. The lack of a decrease in latencies over days with LGDE in concept learning, but not spatial learning, may mean that the drug slows down decision time in difficult, but not easier tasks. This must be evaluated in further studies.

In general, the results of this study confirm previous experimentation (1, 9, 13) concerning the important role of NMDA receptors in spatial learning. The present study also indicates the role of NMDA receptors in concept learning. It seems that concept learning is affected by lower doses of ketamine than those required to disrupt spatial learning. At the dose range tested (120–360 mg/kg), which was found previously to impair a non-spatial task, visuo-tactile discrimination learning (10), LGDE was less effective in disrupting concept or spatial learning. Although it must not be assumed that comparisons between the two drugs imply equivalent dose ranges, these results underline the particular importance of NMDA receptors in spatial learning. However, both NMDA and non-NMDA receptors may be involved in concept learning.

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