Involvement of Hippocampal NMDA and AMPA Receptors in Acquisition, Formation and Retrieval of Spatial Memory in the Morris Water Maze

K.C. Liang, Wai Hon, Yih-Min Tyan and Wen-Lin Liao

Department of Psychology
National Taiwan University
Taipei, Taiwan, Republic of China

Abstract

This study investigated the roles of hippocampal N-methyl-D-aspartate (NMDA) receptors and α-amino-3-hydroxyl-5-methyl-4-isoxazole propionate (AMPA) receptors in acquisition, consolidation and retrieval processes of spatial memory. Male Wistar rats with indwelling cannulae in the dorsal hippocampus received 4 training trials on the Morris water maze for consecutively 6 days. Rats received infusion of the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP5) or the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) into the hippocampus under one of the three schedules: 5 min prior to each daily training session, immediately after each daily training session or 5 min prior to the final testing trial. Pretraining intra-hippocampal infusion of 5.0 μg AP5 retarded acquisition. The same dose of AP5 given after training had little effect but a higher dose (10.0 μg) did slow down progress in the acquisition curve. Pretest infusion AP5 failed to affect memory retrieval. Pretraining intra-hippocampal infusion of 1.0 μg CNQX also impaired acquisition, but posttraining infusion of CNQX at 1.0 or 2.0 μg had no effect. However, pretest infusion of 1.0 μg CNQX markedly impaired retrieval of the already-formed spatial memory. These findings taken together suggest that acquisition in a spatial task involves hippocampal NMDA and AMPA receptors, consolidation of spatial memory involves NMDA receptors and retrieving such memory involves AMPA receptors. (Chinese J. Physiol. 37: 201-212, 1994)

Key Words: hippocampus, NMDA receptors, AMPA receptors, spatial learning, memory consolidation, memory retrieval

Introduction

Substantial evidence implicates the hippocampus in acquisition and retention of spatial information (2,50). Electrophysiology studies have shown in rats or monkeys that a specific class of hippocampal neurons fire in bursts as particular places in a familiar environment or visual field are explored or searched (39,49,58,59). Consistent with these correlative findings, lesions, electrical stimulation or microinfusion of chemicals administered to the hippocampus or its associated structures caused learning and/or memory deficits in spatial tasks (46,48,51,53,54). This preferential role of the hippocampus in processing spatial information has been doubly or triply dissociated from other structures such as the amygdala or striatum which are involved in other forms of learning (21,35,52,55,56).

Long-term potentiation (LTP) is the most prevailing neurophysiological model for learning and memory (6). To induce LTP in the hippocampal CA1 region and dentate gyrus involves activation of N-methyl-D-aspartate (NMDA) receptors (9), although a conjoint contribution from the metabotropic glutamate receptor has recently been suggested (4). On the other hand, α-amino-3-hydroxyl-5-methyl-4-isoxazole propionate (AMPA) receptors which subserve normal glutamate neurotransmission in the hippocampus (41) mediate the augmented synaptic currents in expression of well-established LTP (19, 47). Under certain conditions, NMDA currents are also potentiated afer tetanic stimulation (3), although

梁庚辰、韓 衛、田意民、廖文霖

there is still a debate on their contribution to LTP in natural conditions (26,38).

The above findings have led many studies to examine the role of glutamate receptors in learning and memory, particularly in tasks involving the hippocampal functions (for review, see 36, 37). The most consensus findings are that NMDA receptor antagonists such as 2-amino-5-phosphonopentanoic acid (AP5) administered during acquisition interfere with later retention (42,43,45,60). Disruption of hippocampal LTP is implicated by such findings, supported by the correlation found between LTP and spatial learning (10,24). However, most of these studies administered NMDA blockers either systemically or into the cerebroventricle. Direct infusion of AP5 into the hippocampus was found to impair water maze learning in one study (44), but the high dose and large infusion volume render the specificity in question. To provide definite support for the hypothesis, the issue should be investigated by more refined intra-hippocampal infusion techniques.

Non-NMDA glutamate receptors are also implicated in behavioral plasticity including learning and memory (25). Activating AMPA receptors induces fast EPSP, which could provide the necessary depolarization for unblocking NMDA ion channels. Therefore, suppressing AMPA activation should render NMDA channels more difficult to open and have a deleterious effect on acquisition. Studies examining involvement of amygdala AMPA receptors in affective memory have yielded supportive evidence (16,18). On the contrary, few studies explored the role of hippocampal AMPA receptors in spatial memory and findings were ambiguous. Therefore, this issue deserves further investigation.

Many previous studies administered glutamate antagonists before the training trial. A pretraining treatment could affect retention by its influence on performance factors (33). The effects of NMDA or AMPA antagonists on sensori-motor functions and anxiogenic processes (5,8,13,32,40) have been suspected to contribute to their memory effects (20). Even a pretraining treatment indeed affects learning and memory per se, it could act on either acquisition during learning or memory consolidation starting after learning. The same treatment sometimes exerts opposite influences on these two processes (42), and thus produces conflicting results. Therefore, to resolve the existing ambiguities and clarify the roles of NMDA and AMPA receptors in spatial learning, it is necessary to adopt both pretraining and posttraining treatment regimens in the same study and compare their effects, as what has been accomplished in studying the amygdala (18,28,29).

In contrast to the extensive research on the neurobiological bases of acquisition and memory consolidation, little work has aimed to unravel the neural mechanism underlying memory retrieval. In view of that expression of LTP involves AMPA receptors, blocking these receptors prior to a memory test should hamper memory retrieval. Recent findings in the amygdala support this suggestion (17,23,30), but again little is known for the role of hippocampal AMPA receptors in retrieving spatial memory. In view of the debate whether expression of LTP in nature engages both NMDA and AMPA receptors, evidence concerning whether these two components have behavioral relevance would have great theoretical significance. To resolve this issue, this study compared the effects of pretest intra-hippocampal infusion of AP5 and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a specific AMPA antagonist, on recalling spatial information in the Morris water maze.

Materials and Methods

Subjects

Male Wistar rats weighing 300 to 350 grams were used in this study. After arriving from the breeding centers, they were housed individually in a vivarium maintained at 21° to 25°C with 50% relative humidity. Food and water were available all the time. A 12:12 light:dark cycle was adopted with lights on at 7:00 a.m. throughout the study.

Surgery

One month after arriving, rats were implanted with guide cannulae bilaterally into the dorsal hippocampus. They received i.p. injection of sodium pentobarbital (45 mg/kg). To prevent respiratory congestion, atropine sulfate (0.4 mg/kg) was given 10 min. before the anesthetics. The anesthetized animal was mounted on a DKI-900 stereotaxic instrument. The incisor bar was set at -3.3 mm and the coordinates to implant cannulae into the dorsal hippocampus were AP. -5.5 mm from the bregma, ML. ± 4.7 mm from the midline and DV. -3.5 mm from the dura. The cannula was made of 23 G stainless steel tubing with 0.33 mm inner diameter and 0.63 mm outer diameter. The length of cannulae was 10 mm. Two 0-0 screws serving as anchors were implanted over the right frontal and the left posterior cortices. The whole assembly was affixed on the skull with dental cement. A stylet of 12 mm was inserted into the cannula to maintain patency. Intra-muscular injections of antibiotics (penicillin, 40,000 I.U.) were given at the end of surgery. Rats were kept warm until resurrection. They recuperated for at least two weeks before any behavioral experiments.

Behavioral Task

Rats were subjected to the Morris water maze task approximately two months after the surgery. During this period, all rats were used in another task (the inhibitory avoidance task). The two tasks were separated by three weeks and we found no apparent interference or transfer between the tasks. The Morris water maze was performed in a circular plastic pool (224 cm in diameter, 46 cm in height) located in a room with distinctive visual cues. Water was filled to a depth of 36 cm and a transparent plastic platform (25 × 25 cm, 32 cm in height) was located at the center of a fixed quadrant. Training started by acclimating the rat to the task environment with two days of free-swimming in the pool with no platform. Each session lasted for 2 min and the rat was picked up from the pool by the experimenter. Rats received 4 consecutive daily training trials for the following 6 days. On each trial, the rat was placed into the water randomly from one of the quadrants. The rat had to swim until it climbed onto the platform submerged underneath the water. The time duration from entering the water to climbing onto the platform was recorded and defined as the escape latency. If the rat failed to find the platform by 120 s, it was picked up by the experimenter and placed onto the platform. The rat stayed on the platform for 60 s, which also served as the intertrial interval. At the termination of each day's training, the rat was dried by towels and an electric heater before being replaced into its home cage. On the day following the last training trial, retention was tested by a probe trial. In this test, the rat was allowed to swim for 2 min in the pool without the platform and the time spent in each quadrant was recorded. If the rat remembered the location of the platform, its swimming time should be biased to the quadrant where the target platform was once located during training.

Drugs and Drug Administration

AP5 was obtained from Sigma (St. Louis, USA) and CNQX, a generous gift from Dr. Davis at Yale University, was from Tocris (Bristol, UK). AP5 was dissolved into a specific brain buffer which in 100 ml contained 0.9 g of NaCl, 4.5 ml of 0.2 M Na_2HPO_4, and 0.95 ml of $NaH_2PO_4 \cdot 2H_2O$, which also used for control infusion. To dissolve CNQX, a small amount of 1 N NaOH was added to the drug and then the total volume was brought up by the specific brain buffer to the desired concentration. The pH value of the drug solution was adjusted by HCl to 7.4. The vehicle (Veh) to dissolve CNQX was used for control infusion.

The intra-hippocampal infusion device was constructed as follows: A piece of 0.5 m polyethylene tubing (PE-20, Clay Adams) was connected to a 10 μl Hamilton microsyringe on one end and cemented to a 30 G dental needle on the other. The syringe and the tubing were first filled with distilled water. Drug solutions were then introduced from the injection needle and separated by a tiny air bubble from the distilled water. Drug infusion was administered to a conscious rat shortly before or after the behavioral test. Care was taken to minimize stressing the animal. The rat was gently held and the injection needles were inserted into the cannulae with the stylet removed. To facilitate diffusion of drugs, the infusion needle protruded 1.5 mm beyond the tip of the cannulae. The rat was placed into a small cardboard container for restraining from drastic movement. The drug was infused bilaterally through a microinfusion pump (CMA/100, Carnegie Medicin, Stockholm) at a rate of 0.5 μl. The infusion volume was 1.0 μl for each side of the hippocampus. After infusion, the needle remained in the cannula for an additional minute before withdrawal and the stylet was replaced immediately to prevent back-flow.

Statistical Analysis

Means of the four daily escape latencies were used to represent each day's performance. The acquisition data over the 6 training days were analyzed by repeated measure design two-way (Drug x Day) ANOVAs, with Day as the within subject variable. The retention data on the probe trial during the testing day were analyzed by repeated measure design two-way (Drug x Quadrant) ANOVAs, with Quadrant as the within subject variable. The difference between a particular treated groups and the control group on a particular day was further analyzed by planned comparisons.

Histology Verification

At the conclusion of each experiment, animals were sacrificed with an overdose of sodium pentobarbital (50 mg per rat, i.p.) and perfused through the heart with physiological saline followed by 10% formalin. The brain was then removed, stored in formalin for at least 48 hours. The brains were sectioned (40 μm) and the slices were stained with cresyl violet. Placements of the cannulae were examined by projecting the stained slides onto a brain atlas chart to record locations of cannula tips.

Experiment I: *Effects of Pretraining Intra-Hippocampal Infusion of AP5 or CNQX on Acquisition.*

The first experiment examined the effects of

blocking hippocampal NMDA or AMPA receptors before training on acquisition and retention performance in the Morris water maze. Four groups of rats were trained on the task as previously described. Prior to each daily training session, they received bilateral intra-hippocampal infusions. One group received AP5 at the dose of 5.0 μg, one group received CNQX at the dose of 1.0 μg, while the remaining two groups received Veh for dissolving drugs. The first training trial started 5 min after termination of the intra-hippocampal infusions. Rats were trained for 6 days. On the 7th day, all rats were tested for retention under no influence of drug by giving a probe test trial. The preference bias for the four quadrants was assessed to indicate the strength of memory.

Experiment II: *Effects of Posttraining Intra-Hippocampal Infusion of AP5 or CNQX on Memory Consolidation.*

This experiment investigated the effects of blocking hippocampal NMDA or AMPA receptors immediately after training on acquisition and retention in the Morris water maze. Six groups of rats were trained on the Morris water maze as previously described. Immediately after the 4th trial of each day's session, they received intra-hippocampal injections of AP5 at the dose of 5.0 and 10.0 μg or CNQX at the dose of 1.0 and 2.0 μg or Veh for dissolving either drug. On the 7th day, all rats were tested for retention under no influence of drug in a probe test trial. The preference bias for the four quadrants was assessed to indicate the strength of memory.

Experiment III: *Effects of Pretest Intra-Hippocampal Infusion of AP5 or CNQX on Memory Retrieval.*

This experiment examined the effect of blocking hippocampal NMDA or AMPA receptors during the retention test on retrieving memory which was formed under a normal condition. Four groups of rats were trained as previously described. They received no treatment either before or after each daily training session. On the testing day, rats received intra-hippocampal infusion of 5.0 μg AP5, 1.0 μg CNQX or Veh dissolving either drug. A probe trial was given 5 min after the infusion. The preference bias for the four quadrants, as measured by the swimming time spent in each quadrant, was assessed to indicate the extent that a well-formed memory was retrieved.

Results

Experiment I: *Pretraining Intra-Hippocampal Infusion of AP5 or CNQX Impaired Acquisition in the Morris Water Maze Task.*

The left panel of Figure 1 shows the acquisition curve for the AP5 experiment. The AP5-treated rats had acquisition deficits: Their escape latencies were longer than the Veh-treated rats throughout the learning period. The data were analyzed as previously described. The analysis revealed a significant Drug main effect ($F(1, 19) = 16.42$, $p < 0.01$), indicating that AP5-treated rats learned more slowly than the controls. However, a significant Day effect ($F(5,95) = 24.95$, $p < 0.01$) and a nonsignificant Drug x Day interaction effect suggesting that both groups made progress during the learning period.

According to the figure, the AP5 group appeared to have poorer performance than the controls on the first day of training. Thus, the two groups might have differed from the beginning and the observed effect was not due to the drug treatment. To evaluate this possibility, we analyzed the trial by trial data on the first day with a 2 $\times$ 4 (Drug x Trial) repeated measure design two-way ANOVA, with the number of trials as the within subject variable. Planned comparisons revealed that the AP5 group and the control group did not differ on the first and the second trials, but differed significantly on the third ($F(1, 19) = 6.94$, $p < 0.02$) and the fourth trials ($F(1, 19) = 7.21$, $p < 0.02$). Such results suggested that the two groups were comparable at the beginning of acquisition, but the drug given before training acted quickly and its impairing effect appeared already at late trials of the first training day.

The right panel of Figure 1 shows the retention performance on the probe trials under no drug influence. The controls spent more time in the target quadrant than any other three quadrants, whereas the AP5-treated rats more or less distributed their time evenly in the four quadrants. The data analyses revealed a significant Quadrant main effect ($F(3, 57) = 17.46$, $p < 0.01$) and a significant Drug x Quadrant interactive effect ($F(3, 57) = 10.47$, $p < 0.01$). Further analysis indicated that the controls spent significantly longer time in searching the Target quadrant than the AP5-treated group did ($F(1, 19) = 22.10$, $p < 0.01$).

The left panel of Figure 2 shows the acquisition data for the CNQX experiment. Pretraining intra-hippocampal injection of 1.0 μg CNQX apparently impaired acquisition performance. The data analyses revealed a significant Drug main effect ($F(1, 15) = 9.29$, $p < 0.01$), indicating that the CNQX-treated rats had poorer performance. The Day main effect was significant ($F(5, 75) = 33.93$, $p < 0.01$), in-

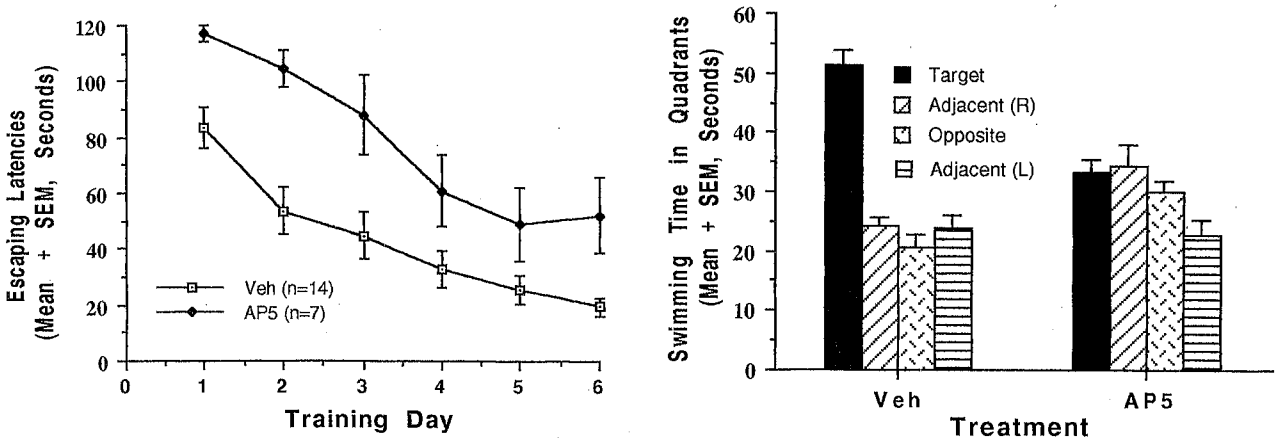

Fig. 1. The left panel shows that the group given pretraining intra-hippocampal infusion of 5.0 μg AP5 was slower in escaping than the Veh group during training. The right panel shows that in the probe-trial retention test, rats given pretraining intra-hippocampal infusion of 5.0 μg AP5 spent less time in the target quadrant.

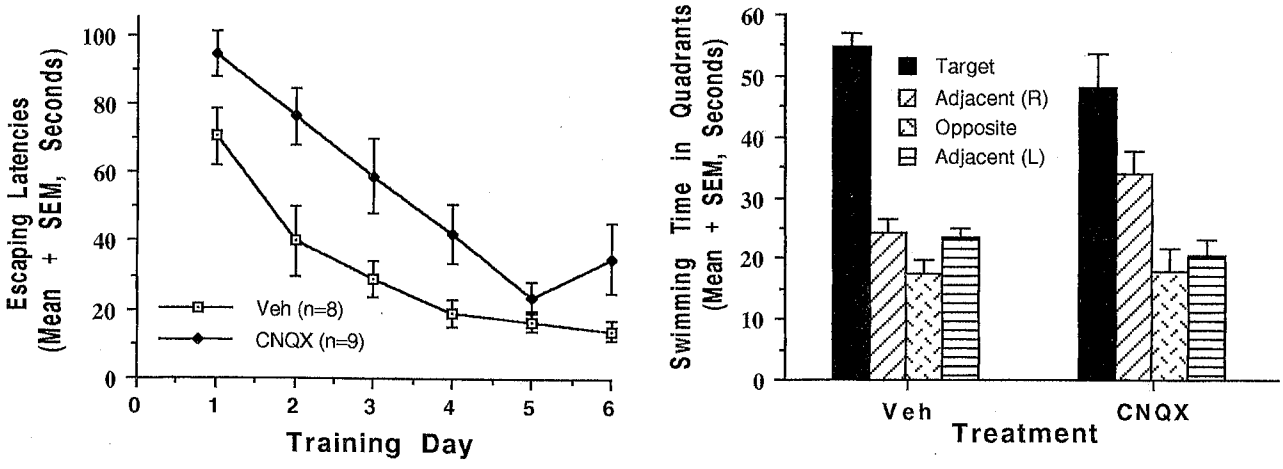

Fig. 2. The left panel shows that the group given pretraining intra-hippocampal infusion of 1.0 μg CNQX was slower in escaping than the Veh group during training. The right panel shows that in the probe-trial retention test, rats given pretraining intra-hippocampal infusion of 1.0 μg CNQX were less discriminative between the target and the right adjacent quadrant.

dicating that both groups improved with increased training. However, the Drug x Day interactive effect was also significant ($F(5, 75) = 11.461$, $p < 0.05$) suggesting that the Veh group and the CNQX group showed different rates in making progress.

The figure shows that the CNQX group appeared to have poorer performance than the controls even from the first day of training. Thus, the observed difference between the two groups might be due to preexisting variance rather than the drug effect. This possibility was evaluated by analyzed the first day data as described in the previous section. Planned comparisons revealed that the CNQX group and the control group did not differ from each other on the first and second trials, but their difference approached significance on the third trial ($F(1, 15) = 3.18$, $p < 0.10$). Such results suggested that the two groups were comparable at the beginning of acquisition, the CNQX effect showed up only in the later trial of the first day.

The right panel of Figure 2 shows the retention performance on the probe test trial for both groups of animals under no influence of the drug. Those rats receiving CNQX during the acquisition phase appeared to be less discriminative in preference among the target and the non-target quadrants. The data were analyzed as previously indicated. The results revealed a significant Quadrant main effect ($F(3, 45) = 51.81$, $p < 0.01$) as well as a significant Drug x Quadrant interactive effect ($F(3, 45) = 2.92$, $p < 0.05$), indicating that the two groups had different pattern of preference among the four quadrants.

Further analysis revealed that the difference in the amount of time spent in the target quadrant approached statistical significance ($F(1, 15) = 3.06$, $p < 0.07$), the CNQX-treated rats spent more time in the quadrant adjacent to the target one ($F(1, 15) = 5.93$, $p < 0.05$), suggesting their memory of the platform might be less precise than the Veh group.

Experiment II: *Posttraining Intra-Hippocampal Infusion of AP5, but not CNQX, Impaired Memory Consolidation in the Morris Water Maze Task.*

The left panel of Figure 3 shows the acquisition curve for the AP5 experiment. Posttraining intrahippocampal appeared to have a discernible impairing effect, especially at the high dose. The data analyses revealed a significant "Day" main effect ($F(5, 100) = 29.20$, $p < 0.001$). While the Drug main effect was not significant, the quadruple trend of the "Drug x Day" interactive effect was significant ($F(2, 20) = 3.75$, $p < 0.05$). Further tests indicated that on the 6th day of training the Veh group had significantly shorter escape latency than the 5.0 μg group ($F(1, 20) = 4.56$, $p < 0.05$) and the 10.0 μg group ($F(1, 20) = 9.57$, $p < 0.01$).

The retention performance on the probe trial is shown in the right panel of Figure 3. The data analyses revealed that the "Quadrant" main effect was significant ($F(3, 60) = 53.67$, $p < 0.001$), indicating that all rats tended to spend more time in the target quadrant. In addition, the "Drug x Quadrant" interactive effect was also significant ($F(6, 60) = 2.44$, $p < 0.05$), reflecting that while the Veh group and the 5.0 μg group spent more time in the target quadrant, the 10.0 μg group distributed the swimming time more evenly among the four quadrants. Further tests revealed that the 10.0 μg group spent less time in the target quadrant than the Veh and the 5.0 μg group did ($F(1, 20) = 5.03$ & 5.67, respectively, $p < 0.05$).

The left panel of Figure 4 shows the learning curve for the CNQX experiment. Posttraining intrahippocampal injection of 1.0 or 2.0 μg CNQX had little effects on performance during the training phase. The data analyses failed to find a significant Drug main effect or Drug x Day interactive effect. Only the Day main effect reached statistical significance ($F(5, 130) = 38.1$, $p < 0.001$). Such results suggested that both the Veh group and the CNQX group acquired the task at approximately the same rate.

The right panel of Figure 4 indicates that on the probe test trial, the three groups appeared to have the same pattern of preference among various quadrants. The data showed that only the "Quadrant" main effect was significant ($F((3, 78) = 51.6$, $p < 0.001$), suggesting that both groups spent more time in the target quadrant.

Experiment III: *Pretest Intra-Hippocampal Infusion of CNQX but not AP5 Impaired Memory Retrieval in the Morris Water Maze Task.*

The groups did not differ during the training phase in which no drug treatment was given. The left panel of Figure 5 shows the amount of time spent in each of the four quadrants during the probe trial for the AP5 experiment. No effect of AP5 on the distribution of swimming time was apparent. A 2 × 4 (Drug x Quadrant) repeated measure two-way ANOVA revealed a significant Quadrant main

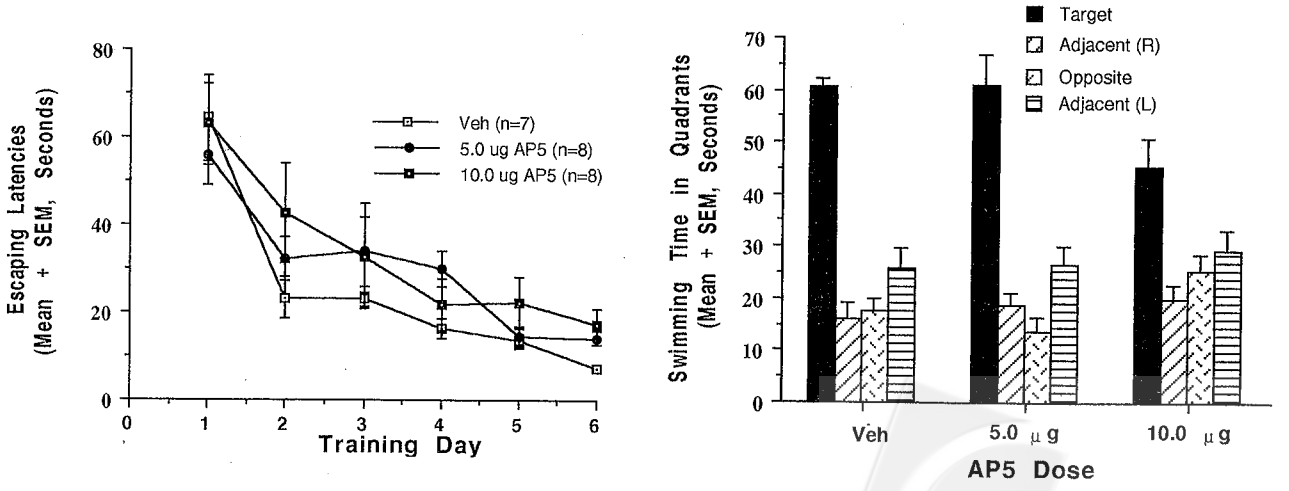

Fig. 3. The left panel shows that the groups given posttraining intra-hippocampal infusion of 5.0 and 10.0 μg AP5 were slower in escaping than the Veh group on the sixth day of training. The right panel shows that in the probe-trial retention test, rats given posttraining intra-hippocampal infusion of 10.0 μg AP5 spent less time in the target quadrant.

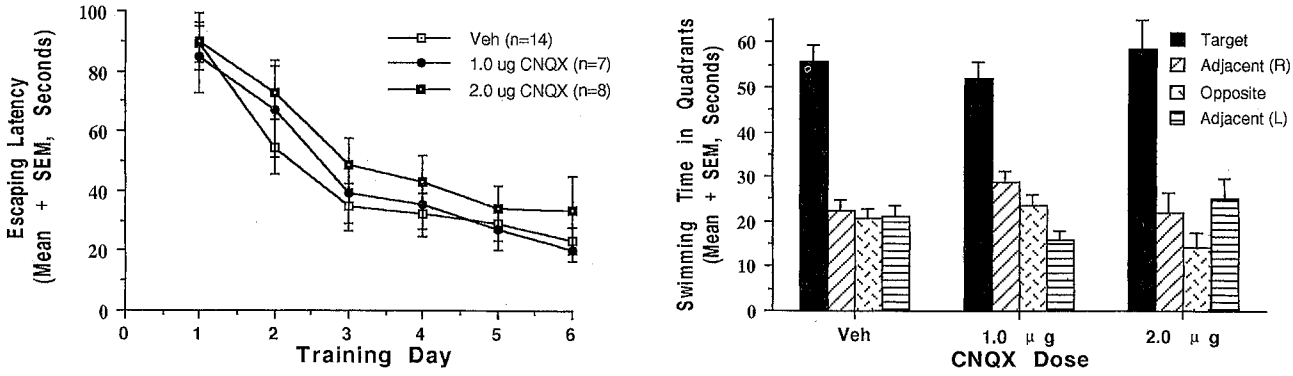

Fig. 4. The left and right panels show the lack of effects of posttraining intra-hippocampal infusion of 1.0 or 2.0 μg CNQX on performance during the acquisition phase and in the probetrial retention test, respectively.

effect ($F(3, 63) = 33.68$, $p < 0.01$), indicating that both groups spent more time in the target quadrant. Neither the Drug main effect nor the Drug x Quadrant interactive effect was significant, suggesting that pretest intra-hippocampal injection of AP5 did not affect retention performance by impeding memory retrieval.

The right panel of Figure 5 shows retention data for the two groups receiving pretest intra-hippocampal infusion of Veh or CNQX. In contrast to the lack of effect of AP5, CNQX given prior to the probe trial in the retention test clearly impaired retention performance by reducing the time spent in the target quadrant. The data analyses revealed a significant Quadrant main effect ($F(3, 63) = 31.73$, $p < 0.01$) and a significant Drug x Quadrant interactive effect ($F(3, 63) = 5.88$, $p < 0.01$), suggesting the pattern of time distribution among the four quadrants were different for the two groups. Further analyses indicated that the CNQX treated rats spent much less time in the target quadrant than the controls ($F(1, 21) = 19.02$, $p < 0.01$), indicating that intra-hippocampal infusion of CNQX impaired retrieval of the target location from memory.

Given that pretest intra-hippocampal infusion of CNQX impaired memory retrieval, pretraining intra-hippocampal injection of CNQX should have a greater effect on acquisition performance in the first trial in which retrieval from memory formed on the day before was necessary. To test this prediction, we re-analyzed the trial by trial CNQX results of Experiment I. We calculated Trial 1 through Trial 4 escape latencies across Day 2 to Day 6 (Day 1 was excluded because it had no bearings on retrieving long-term memory). The resulted data, as presented in the Figure 6, were subjected to 2 × 4 (Drug x Trial) repeated measure design two-way ANOVA, with Trial as the within subject variable. The most relevant finding was that the Drug x Trial interactive effect was significant ($F(3, 45) = 5.66$, $p < 0.002$), indicating CNQX had differential effects on various trials. This interactive effect was due to greater difference between the Veh and the CNQX groups on Trial 1 than on subsequent trials in average ($F(1, 15) = 14.65$, $p < 0.002$). To test whether the huge difference on Trial 1 from Day 2 to Day 6 merely reflected the difference in learning on the previous day (because pretraining CNQX impaired acquisition as shown by results of Exp. I), the data on the last trial from Day 1 to Day 5 were collapsed and designated as Trial 0 to serve as the initial baseline for the Trial 1 from Day 2 to Day 6. An analysis of Trial 0 and Trial 1 data by a two-way repeated measure design ANOVA revealed that, most importantly, the Drug x Trial interactive effect was significant ($F(1, 15) = 7.72$, $p < 0.05$), suggesting that the difference between the Veh and the CNQX groups was greater for Trial 1 than for Trial 0. Therefore, difference in the extent of acquisition could not completely account for the much poorer first trial performance from Day 2 to Day 6 of the pretraining CNQX-treated rats.

Histology

The photomicrograph of typical hippocampal cannula tracts in a representative animal is shown in Fig. 7. All of cannulae were found to locate in dorsal part of the hippocampus.

Discussion

The major findings of the present study could be recapitulated as follows: First, pretraining intra-hippocampal infusion of AP5 or CNQX impaired acquisition/retention in a Morris water task. Second, posttraining intra-hippocampal infusion of AP5 but not CNQX had a slight, nonetheless significant, effect on acquisition/retention in this task. Third,

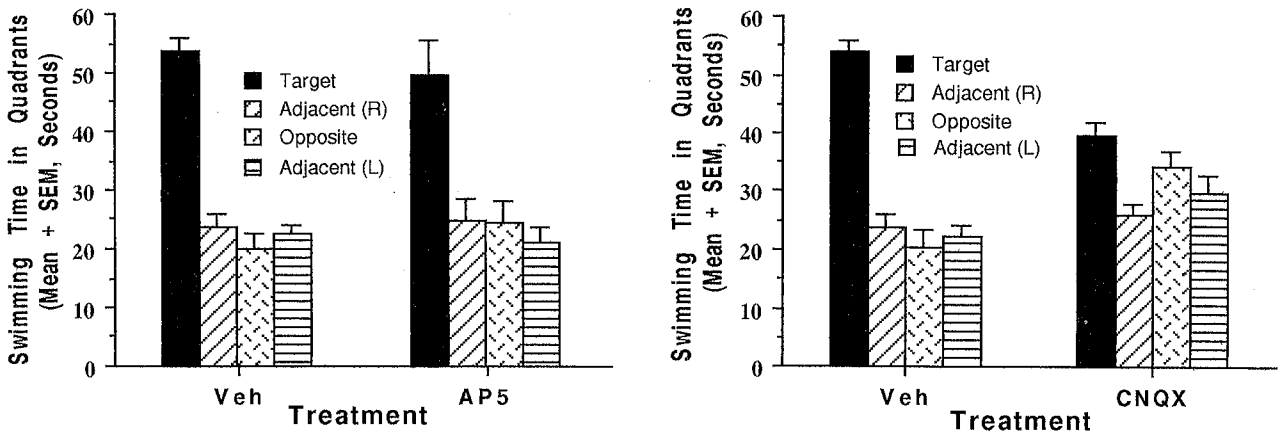

Fig. 5. The left panel shows lack of effects of pretest intra-hippocampal infusion of 5.0 μg AP5 on performance in the probe trial memory test. The right panel shows that pretest intra-hippocampal infusion of 1.0 μg CNQX decreased the time spent in the target quadrant during the probe-trial memory test.

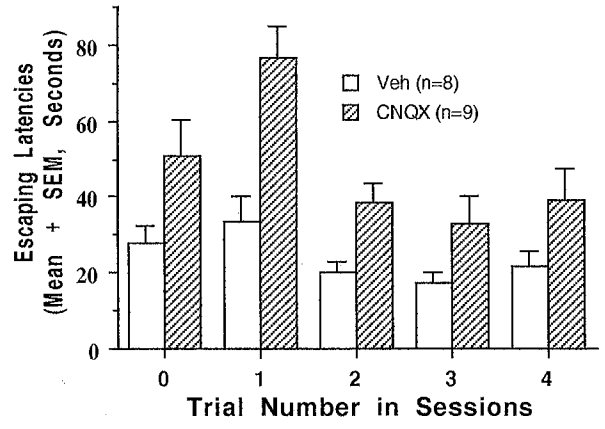

Fig. 6. Pretraining intra-hippocampal infusion of 1.0 μg CNQX had a greater effect on the first trial than on subsequent trials in each daily session. Trial 1 to Trial 4 represent escape latencies collapsed from Day 2 to Day 6 for the first to the fourth trial, respectively. Trial 0 represents the escape latencies collapsed for the last trial of Day 1 to Day 5 serving as the baseline.

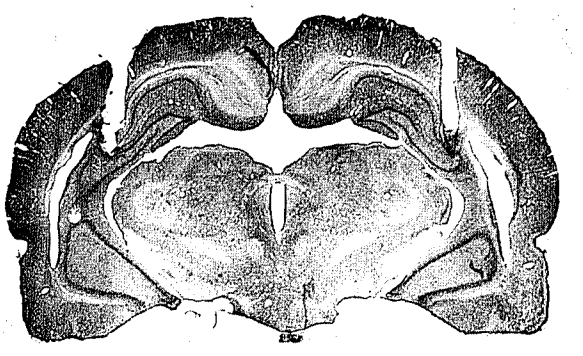

Fig. 7. Photomicrograph of typcial hippocampal cannula tracts in a representative rat.

pretest intra-hippocampal infusion of CNQX but not AP5 impaired recall of a well learned spatial response. These findings, taken together, suggest that acquisition of spatial information engages both NMDA and AMPA receptors in the hippocampus. Consolidation of such information in the long-term storage involves NMDA receptors, whereas retrieval of such information after consolidation involves AMPA receptors.

The present results that pretraining intra-hippocampal infusion of AP5 impaired acquisition/retention in the Morris water maze replicate and extend the findings by Morris and his colleagues (44). In their study, a single infusion of 2 μl of 50 mM AP5 (approximately 20 μg) into the dorsal hippocampus before training impaired learning in the Morris water maze. Our effect was observed under a lower dose (5.0 μg) and smaller infusion volume (1 μl) which improved the anatomical and pharmacological specificities. We have previously shown that the behavioral effect of 5.0 μg AP5 can be abolished by 0.5 μg of NMDA, excluding any possible contribution of non-NMDA action induced by AP5 to the observed effect (31). Findings from this and other laboratories found that drug diffusion was confined within the hippocampus with this infused volume (15,27). AP5 given before training is not likely to affect acquisition/retention by acting on performance factors, because AP5 given prior to a memory test failed to affect expression of a well-learned spatial habit as shown in Experiment III. Further, the deficit observed in the acquisition phase persisted in the probe trial: Under no influence of drugs, rats previously receiving AP5 still spent less time in the

target quadrant. Thus, the AP5-treated rats indeed acquired and remembered poorly.

In contrast to the previous findings (42), this study found that immediate posttraining intra-hippocampal infusion of 10.0 μg AP5 caused significant deficits during the acquisition phase and in the retention test. AP5 is not likely to be around 24 hrs after its administration and to exert a proactive influence in the memory test; otherwise, the pretest AP5 treatment in Experiment III should have produced a greater effect. Further, when retention was tested in the probe trial, no drug was given. Therefore, the present results suggest that posttraining intra-hippocampal infusion of AP5 impaired memory consolidation processes. Previous studies unable to show a posttraining AP5 effect mostly administered the blocker either systemically or into the cerebroventricle. It should be noted that the Morris water maze generally has multiple trials in daily training sessions. Posttraining treatments are given at the end of each session instead of each trial. Memory consolidation processes ensuing after the first trial may have progressed substantially at the time when subjects receive treatments. Thus, only drugs applied directly to the hippocampus could reach the relevant synapses with sufficient concentrations in the time window when the trace is still susceptible for modification. Even direct infusion of AP5 after four trials of training may still allow the consolidation process to proceed normally to some degrees. This could partly account for why the posttraining effect of AP5 was smaller and only appeared at a higher dose (10 μg rather than 5 μg). Further, AP5 infused before training might affect both acquisition and memory consolidation phases, but AP5 infused after training could only affect the latter. Accordingly, if single-trial training sessions were employed, we might have observed a greater effect of posttraining infusion of AP5. This possibility should be explored in the future.

The present findings appear to be incompatible with the physiological evidence that AP5 applied after tetanic stimulation failed to modify already established LTP (9). However, given that the magnitude of potentiation is cumulative (11), robust memory traces may be formed only after multiple trains of tetanic stimuli. Thus, AP5 administered after training could presumably prevent reverberatory neural activity continuing after cessation of training (14) from inducing LTP. Such conjecture is consistent with recent findings that hippocampal place neurons were reactivated during the sleep episodes after spatial learning (63). The present results and previous ones that posttraining infusion of dynorphin impaired retention in the same task (34) may provide the behavioral analog for a consolidation phase in establishing long-lasting LTP, which was recently suggested by neurophysiology experiments (1).

The present results showed that pretraining intra-hippocampal infusion of CNQX also impaired learning and memory in the Morris water maze. A recent study reported that intra-amygdala infusion of CNQX was anxiolytic and reduced sensitivity for noxious stimuli (40). In our laboratory, we have found that intra-hippocampal infusion of CNQX had no effect on visual discrimination and reversal learning in the Morris water maze (15). Therefore, the effect of CNQX given before training could hardly be due to its influences on sensori-motor or motivational factors. These results demonstrate, probably among the first to the best of our knowledge, a role of hippocampal AMPA receptors in acquiring spatial information. This and other laboratories have previously shown that amygdala AMPA receptors are critically involved in acquiring affective information (17,23,31). All these findings are consistent with a notion that the membrane depolarization necessary for opening NMDA channels is brought about by AMPA receptors activation, presumably due to glutamate released by training.

In contrast to the significant effect of posttraining AP5 on memory consolidation processes, posttraining intra-hippocampal infusion of 1.0 or 2.0 μg CNQX failed to affect acquisition/retention in the present task. Conversely, we and others have previously shown that posttraining intra-amygdala infusion of 0.3 to 1.0 μg CNQX impaired retention in the inhibitory avoidance task (16,18,31). We did attempt to use a higher dose of CNQX but in vain due to dissolving problems and frequent incidents of convulsions in rats such treated. While posttraining intra-hippocampal infusion of CNQX might affect Morris water maze learning at other doses infused into a more sensitive hippocampal sector (57), the present findings nonetheless hint that hippocampal AMPA receptors play a less important role than NMDA receptors in consolidating newly acquired spatial information. A speculated account for such findings is that once AMPA receptors have provided the initial depolarization for opening NMDA channels in the early phase of training, they are no longer needed if the multiple-trial training continuously releases glutamate, because the NMDA-induced slow EPSPs may be large enough after summation to provide the necessary depolarization.

The present study found that pretest intra-hippocampal infusion of AP5 did not affect performing an established spatial habit in the Morris water maze. Consistent with similar findings of previous studies administering NMDA antagonists into either the periphery or cerebroventricle (22), these findings suggest that the NMDA component of hippocampal

synaptic currents, even being potentiated by tetanic stimuli under certain conditions, may not participate normally in coding and expressing spatial memory. normally in coding and experesing spatial Previous evidence has also excluded a role of amygdala NMDA receptors in expressing affective memory (22,28,29). On the contrary, pretest intra-hippocampal infusion of CNQX impaired retrieval of the already-formed spatial memory. Such finding is consistent with the physiological evidence on LTP expression and suggests that AMPA receptors play a role in utilization of already-acquired spatial information. Amygdala AMPA receptors also subsume a similar role according to findings in this and other laboratory (17, 21,30).

Re-analysis of data from Experiment I provides further support for the role of AMPA receptors in memory retrieval. Pretraining intra-hippocampal infusion of CNQX had more detrimental effects on performance in the first trial than other three trials from Day 2 to Day 6. It is conceivable that in the first trial of a session, a rat has to retrieve information about the platform location from long-term memory formed on the previous day. However, once a rat has reached the target, either directed by memory or by trial and error, it may count on working memory in the subsequent trials of the same session. Thus, pretest infusion of CNQX should produce the severest deficit in the first trial of a multiple-trial test. This conjecture has been verified by a later study (15). Such findings, taken together, suggest that hippocampal AMPA receptors are involved in retrieving information from the long-term storage but not in utilizing information already available to the working memory. The latter part of this suggestion appears to be in conflict with the evidence that the hippocampus subserves working memory in the radial arm maze task (50, 51). However, working memory may be mediated by hippocampal functions other than AMPA receptors. Conversely, working memory may depend on the hippocampus more in tasks involving alternating responses or win-shift strategies (62), such as the radial arm maze or delayed nonmatching to sample task. These possibilities should be evaluated in further research.

The present results add to the cumulative evidence supporting a widely conceived model that hippocampal LTP subserves the neural basis underlying spatial learning and memory: Blocking either NMDA or AMPA receptors, which are necessary for or involved in LTP induction, erodes acquisition/retention of spatial information. Blocking AMPA receptors implicated in LTP expression, impedes retrieval of spatial memory. However, it deserves our attention that in both Experiment I and II, rats treated with either CNQX or AP5 during training still made very significant progress across the sessions, as attested by the highly significant Day main effect. Many other studies have also shown that treatments compromising NMDA-dependent LTP mechanisms retarded but not abolished spatial learning (45,61). While the possibility that the treatments fail to completely eliminate LTP is hard to rule out, the present results raise serious questions on the notion that NMDA-dependent LTP is the exclusive neural substrate of spatial learning and memory. In performing the Morris water maze, brain regions other than the hippocampus are also activated based on 2-DG uptake or c-fos immunoreactivity (12). Further, under certain circumstances, relatively normal retention could be resulted in rats receiving high doses of AP5 (31). Such memory must be mediated by neural processes independent of NMDA-related plasticity. Neural bases other than LTP underlying behavioral plasticity related to either spatial learning or learning in general should be seriously explored.

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