Chinese Journal of Physiology 36(1): 47-56, 1993

Involvement of Amygdala N-Methyl-D-Asparate Receptors in Long-Term Retention of an Inhibitory Avoidance Response in Rats

K.C. Liang, Ming-Hung Lin and Yih-Min Tyan

Department of Psychology
National Twawan University
Taipei, Taiwan 10764
Republic of China

Abstract

This study examined the involvement of amygdala N-methyl-D-aspartate (NMDA) receptors in long-term retention of an inhibitory avoidance response. Rats bearing chronic cannulae implanted into the basolateral amygdala were trained on a one-trial inhibitory avoidance task and tested for retention 21 days later. They received intra-amygdala injections of vehicle (Veh) or 0.25, 1.25 or 5.0 μg of a competitive NMDA antagonist--2-amino-5-phosphonopentoic acid (AP5) either 5 min before training, immediately after training or 5 min before testing. Results indicated that pretraining intra-amygdala injections of AP5 at all doses impaired retention performance profoundly. Intra-amygdala injections of AP5 immediately after training caused a dose-dependent retention deficit: 0.25 μg induced no deficit and 5.0 μg induced a great deficit. Immediate posttraining intra-amygdala injections of a non-competitive NMDA antagonist MK-801, also impaired retention but MK-801 given 2 hrs after training had no significant effect. In contrast to the marked amnestic effect produced by pre- or posttraining intra-amygdala injections of AP5, intra-amygdala injections of AP5 given just before retention tests had no discernible effect on retention performance. The retention deficit induced by pretraining intra-amygdala injections of 1.25 μg AP5 was ameliorated completely by N-methyl-DL-aspartate (0.25 μg), but also partially by norepinephrine (0.2 μg) infused into the amygdala immediately after training. However, posttraining infusion 0.2 μg norepinephrine failed to attenuate significantly the amnestic effect induced by 5.0 μg AP5. These findings, taken together, suggest that NMDA receptors in the amygdala are normally involved in memory formation processing of affective experience. (Chinese J. Physiol. 36: 47-56, 1993).

Key Words: amygdala, NMDA receptors, norepinephrine, avoidance learning, long-term memory.

Introduction

The amygdala is implicated in memory processing for affective experience (2, 12): Lesions or stimulation of the amygdala or its afferent-efferent pathways impaired acquisition or retention in fear-motivated learning tasks (37, 40). Treatments that alter noradrenergic, opioid, cholinergic or GABAergic functions of the amygdala could enhance or impair retention if applied immediately after the training experience, but had no effect if applied hours after training on the inhibitory avoidance task – a typical fear-motivated task (46). Such findings are interpreted as that memory consolidation processes involve functioning integrity of various neurochemical systems within the amygdala (45). However, exactly how these amygdala neurochemical systems participate the memory functions is yet to be elucidated.

Long-term potentiation (LTP) is a rapid enhancement of synaptic efficacy after brief tetanus stimulation. In the hippocampal CAl region, dentate gyrus as well as the visual cortex, induction of LTP depends on activation of NMDA receptors (41), although NMDA-independent forms of LTP may also exist in other brain regions (26). Consistent with a prevailing notion that LTP may serve as a physiological model for the neural plasticity underlying learning and memory (4), there is ample evidence

梁庚辰、林明鴻、田意民
Send all correspondence to: Dr. Keng Chen Liang, Department of Psychology, National Taiwan University, 1 Roosevelt Rd., Sec. 4 Taipei, Taiwan 10764 R.O.C.

showing involvement of NMDA receptors in learning and memory: Systemic or intra-cerebroventricular (icv) injections of NMDA receptor antagonists around training impaired acquisition/retention performance in various learning tasks including those motivated by fear (1).

The amygdala receives excitatory amino acid projections from the cerebral cortex and thalamic regions (3,5,33). These projections have been implicated in memory functions of the amygdala (17,55). High densities of NMDA and non-NMDA receptors are present in the basolateral and lateral amygdaloid nuclei (48). Stimulation of afferents to these nuclei activates in amygdala neurons excitatory postsynaptic potentials containing both NMDA and non-NMDA components (18). Several studies have demonstrated LTP in the basolateral amygdala complex either in vitro or in vivo (9,10). A recent study further showed that LTP induced in amygdaloid slices was NMDA-dependent (17).

In view of the above evidence, NMDA antagonists injected into the periphery or ventricle should affect fear-motivated learning by acting on amygdala NMDA receptors. Indeed, several recent studies have shown that injection of NMDA blockers including 2-amino-5-phosphonopentoic acid (AP5) into the amygdala basolateral nucleus, shortly before fear training, impaired acquisition as well as extinction of conditioned fear-potentiated startle to a visual or an auditory stimulus (7,15,47), consistent with a view that extinction involves forming new inhibitory association. In a multiple-trial inhibitory avoidance task, Kim and McGaugh (32) demonstrated that pretraining intra-amygdala injections of various NMDA antagonists impaired retention. Recently, two studies reported that posttraining intra-amygdala infusions of AP5 impaired a one-trial inhibitory avoidance task when given immediately after training (25,36), which suggested that memory consolidation processes per se was affected.

Memory for emotional events generally could last for a long time. However, all the above studies tested retention only 1 or 2 days after training. Literature has documented that the nature of a memory trace may alter over time since its formation, as revealed by differential susceptibility of retention after various intervals to posttraining or pretesting treatments (13,52). Previous findings from this laboratory also showed that retention in the inhibitory avoidance task had diminishing susceptibility to interference of pretest intra-amygdala injections of lidocaine over a 21-day period (35). To evaluate whether amygdala NMDA receptors, and by inference LTP, are involved in this very long-term retention of affective experience, it is necessary to show whether the intra-amygdala injections of AP5 produced similar effect on 21-day retention as for 1- or 2-day retention. Further, several previous studies showed that pretest intra-amygdala injections of AP5 did not affect expression of already-formed memory (7,32,36), which was puzzling given that the NMDA-component of amygdala EPSP, with a late development, was significantly potentiated by tetanus stimulation (21). Potentiation of the amygdala NMDA component, while not involved in 1 or 2 day retention test, may have a role during recollection of the affective experience after a long retention period. We therefore examined the effect of AP5 given prior to a long-term retention test.

Activating NMDA receptors in the hippocampus or amygdala caused release of NE and DA (27,51, 53,57). Recent evidence has indicated that isoproterenol produced a sustained enhancement of excitatory postsynaptic potentials in amygdala slices (20). Intra-amygdala infusions of NE or its β-antagonists had pronounced effects on retention (38), which have been taken as evidence that NE in the amygdala subserved a memory modulatory function (45). Therefore, the amnestic effect of AP5 injected into the amygdala may be mediated, at least partially, by blocking NE release. Although evidence contradicting such a suggestion has been reported that the memory facilitating effect of NE in the hippocampus was abolished under NMDA blockade (34), consistent with the findings that NE-induced long-term potentiation in the dentate gyrus was NMDA-dependent (6,58), possible interactions between NMDA and noradrenergic activation in affecting memory processes have not been investigated in the amygdala. The present study was also designed to address this question.

Materials and Methods

Animals

Male albino Sprague-Dawley rats, 100 days old, obtained from breeding centers of National Yang-Ming Medical College as well as National Defense Medical College, were used in the present study. Upon arrival, they were housed individually in air-conditioned and temperature-controlled rooms with free access to food and water. Throughout the study, a 12/12 h light/dark cycle was adopted with lights on at 7:00 a.m.. Behavioral tests were always performed between 1:00 p.m. and 5:00 p.m.

Surgery

Three to four weeks after arriving, rats were anesthetized with intraperitoneal injections of sodium pentobarbital (50 mg/kg). To prevent respiratory congestion, atropine sulfate (400 μg/kg) was given

30 minutes before anesthetics. After being shaved on the head, the anesthetized animal was mounted on a stereotaxic instrument (DKI-900). A midline incision was made to expose the skull, and two cannulae made of 23 gauge stainless steel tubing were implanted bilaterally into the dorsal surface of the amygdala (coordinates: A.P. −2.5 mm from the bregma, M.L. ± 4.7 mm from the midline, D.V. −6.0 mm from the surface of the skull). Two jewelry screws were implanted over the right frontal and the left posterior cortices serving as anchors. The whole assembly was affixed on the skull with dental cement.

Intra-muscular injections of antibiotics were given at the end of each surgery. Rats were kept warm until resurrection from anesthesia. Animals were allowed to recuperate from the surgery for at least two weeks before any behavioral tests.

Behavioral Tasks

The inhibitory avoidance apparatus was a trough-shape alley divided into two compartments described elsewhere (40). The safe compartment was lit by a 20-Watt light bulb and the shock compartment was dark. The rat was placed into the lit compartment facing away from the door. As the rat turned around, the door was opened. As the rat stepped into the dark compartment, the door was closed and an inescapable footshock (1.75 mA/1s) was administered through the floor. This intense training footshock was employed to insure that the control group could show good memory in a 21-day retention test which would allow easy demonstration of any possible amnestic effect. The shock was administered by a constant current shocker connected to a timer (Lafayette Instruments, Model 80240 and Model 58010, Lafayette). The shock intensity was determined as the root mean square of the sine wave alternating current.

After administration of the shock, the rat was retrieved from the alley and returned to his home cage. In the retention test given 21 days later, the rat was reintroduced into the alley and its latency to step into the dark compartment was taken as a retention score. If the rat did not step through in 10 minutes, the test trial was terminated and a ceiling score of 600 (seconds) was assigned.

Drug Administration

Norepinephrine, DL-2-amino-5-phosphonopentoic acid (AP5) and N-methyl-DL-aspartate (NMDA) were from Sigma (St. Louis, MO), (+)-MK-801 was from Research Biochemical Incorporated (Natick, MA). Durgs were 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 served as the vehicle (Veh) for control injections. The pH value of AP5 solutions was adjusted to 7.4 by NaOH. The intra-amygdala injection device was constructed as follows: A piece of 0.5 meter polyethylene tubing (PE-20, Clay Adams) was connected to a 10 μl Hamilton microsyringe on one end and cemented to a 30 gauge 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.

Intra-amygdala injections of drugs were 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 injection needle protruded 1.5 mm beyond the tip of the cannulae. The rat was then placed into a small cardboard container for restraining from drastic movement. Bilateral intracranial injections were administered through a microinjection pump (CMA/100, Carnegie Medicin, Stockholm) at a rate of 0.5 μl per minute. A total volume of 0.5 μl was infused into each site in each injection. After the injection, the injection needles were kept in the cannulae for an additional minute before withdrawn and the stylet was replaced immediately to prevent back flow. Behavior training or testing commenced 5 min after the stylet was replaced.

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 mm) with a microtome. The brain slices stained with cresyl violet. Placements of the cannulae were examined by projecting the stained slides onto a brain atlas chart and recording the location of the cannula tips on the chart.

Experiment I: Effects of Pretraining Intra-Amygdala Injections of AP5 on Retention.

The first experiment examined the effect of pretraining intra-amygdala injections of AP5 on acquisition/retention performance in the inhibitory avoidance task. Four groups of rats received bilateral intra-amygdala injections of Veh, or 0.25 μg, 1.25 μg or 5.0 μg of AP5. All rats were trained 5 minutes after termination of the intra-amygdala injections of

Veh or AP5.

Experiment II: Effects of Posttraining Intra-Amygdala Injections of AP5 on Retention.

This experiment investigated the effect of posttraining intra-amygdala injections of AP5 on retention. Immediately following training and before being replaced back to the home cage, four groups of rats received intra-amygdala injections of Veh or AP5 at the doses of 0.25, 1.25 or 5.0 μg. Retention was tested 21 days later.

Experiment III: Effects of Posttraining Intra-Amygdala Injections of MK-801 on Retention.

To evaluate the generality that NMDA receptors might still be activated shortly after training and involved in memory proecssing, the third experiment examined the effect of posttraining intra-amygdala injection of a non-competitive antagonist MK-801 on memory. Five groups of rats were trained. Immediately after training, four groups received intra-amygdala injections of Veh, 0.05, 0.25 or 1.0 μg MK-801. The extra group received a delayed injection of 0.05 μg MK-801 given 2 h after training.

Experiment IV: Effects of Pretest Intra-Amygdala Injections of AP5 on Retention.

This experiment investigated whether intra-amygdala injection of AP5 shortly before the retention test would affect the retrieval process. Rats were trained on the inhibitory avoidance task but received no treatment either before or after training. They were tested for retention 21 days later. Intra-amygdala injections of Veh, 0.25 μg, 1.25 μg or 5.0 μg of AP5 were administered 5 min prior to the retention test.

Experiment V: Attenuation of the AP5 Amnestic Effect by NMDA or NE.

To explore whether the amnestic effect of AP5 injected into the amygdala was due to blockade of NMDA receptors or due to some not-yet specified actions of AP5, we examined the influence of posttraining injections of N-methyl-DL-aspartate (NMDA) or norepinephrine (NE) on the amnestic effect of AP5 injected before training. As a competitive agonist of the receptors, NMDA should attenuate the AP5 amnestic effect. Further, if AP5 impaired memory by blocking NE release due to stress during training such as the intense training footshock, an enhancing dose of NE should normalize the retention in rats treated with AP5. Six groups of rats were trained as described previously. They received one of the following pretraining/posttraining treatments administered to the amygdala: Veh/Veh, 1.25 μg AP5/Veh, 1.25 μg AP5/0.25 μg NMDA, 1.25 μg AP5/0.2 μg NE, 5.0 μg AP5/Veh, 5.0 μg AP5/0.2 μg NE. Retention was tested 21-days later.

Results

Experiment I: Pretraining Intra-Amygdala Injections of AP5 Impaired Retention.

As indicated in the Method section, the distribution of the retention scores in the present study was truncated at 600. Consequently, *medians* and *interquartile ranges* were used to represent, respectively, the central tendency and the dispersion of the data, and non-parametric statistics (Kruskal-Wallis analysis of variance and Mann-Whitney U-tests) were used to analyze the data.

The 21-day retention performance is shown in Fig. 1. Pretraining intra-amygdala injections of AP5 induced a pronounced retention deficit. A Kruskal-Wallis one-way analysis of variance revealed a significant overall difference among various groups ($H(3) = 17.21$, $p < 0.01$). Paired comparisons by Mann-Whitney two-tailed U-tests indicated that the Veh group had significantly better retention scores than the groups receiving 0.25 μg, 1.25 μg or 5.0 μg of AP5 ($U = 22.5$, 24 & 4; $p < 0.01$). The group receiving 5.0 μg AP5 showed significantly lower retention scores than the group receiving 0.25 μg ($U = 17.5$; $p < 0.01$), while groups receiving 5.0 or 1.25 μg did not significantly differ from each other.

Experiment II: Posttraining Intra-Amygdala Injections of AP5 Impaired Retention.

The retention performance of various groups is

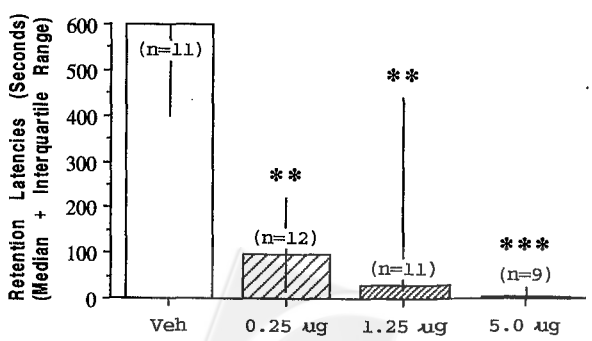

Fig. 1. Effects of pretraining intra-amygdala of AP5 on retention performance. *$p < 0.05$, **$p < 0.02$, ***$p < 0.001$ different from the Veh group.

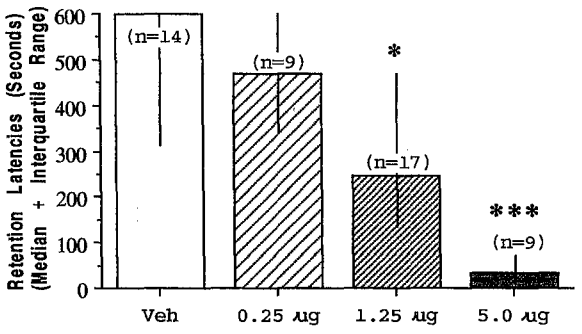

Fig. 2. Effects of posttraining intra-amygdala injection of AP5 on retention performance. *$p<0.05$, ***$p<0.001$ different from the Veh group.

shown in Fig 2. Posttraining intra-amygdala injections of AP5 impaired retention in a dose-dependent manner with 5 μg producing the greatest deficit and 0.25 μg producing little effect. A Kruskal-Wallis one-way analysis of variance revealed a significant overall difference among the groups ($H(3)=24.51$, $p<0.01$). Paired comparisons by Mann-Whitney two-tailed U tests indicated that the retention scores of the Veh group were significantly better than those of groups receiving AP5 at the dose of 1.25 μg or 5.0 μg ($U=50$ & 1, $p<0.05$ & 0.001; respectively). The group receiving 0.25 μg had retention scores not significantly different from the Veh group. Further, the 5.0 μg group had significantly lower retention scores than the 1.25 μg group and the 0.25 μg group ($U=4$ and 0, respectively, $p<0.001$), while the latter two were not significantly different from each other.

Because the control performance in Exp. I & II was comparable, it is possible to evaluate the relative effectiveness of pretraining and posttraining treatments. Paired comparisons between the retention scores in Exp. I & II indicated that rats receiving 0.25 μg AP5 after training had significantly better retention than rats receiving the same dose of AP5 before training (1-day vs 21-day, $U=12.5$, $p<0.01$). A similar trend was also found in rats receiving 1.25 μg AP5, but the difference only approached statistical significance (1 day vs 21-day, $U=49$, $0.05<p<0.10$). Rats receiving 5.0 μg AP5 either before or after training showed no significant difference in retention performance.

Experiment III: *Posttraining Intra-Amygdala Injections of MK-801 Impaired Retention.*

Retention performance is shown in Fig. 3. Immediate posttraining intra-amygdala injections of MK-801 produced a robust time-dependent retention deficit: MK-801, given immediately after training, severely impaired retention at all doses. However, an effective dose of MK-801 had no effect if given 2 hrs after training. A Kruskal-Wallis one-way analysis of variance indicated a significant overall difference among the groups ($H(4)=19.29$, $p<0.01$). Paired comparisons by Mann-Whitney U-tests revealed that groups receiving 0.05, 0.25 or 1.0 μg MK-801 had significantly lower retention scores than the Veh-injected group ($U=4$, 18 & 36, respectively; $p<0.01$). While the smallest dose (0.05 μg) of MK-801 tended to have the poorest retention, the differences among the three drug-treated groups were not significant. Retention scores of rats receiving delayed injection of 0.05 μg MK-801 were not significantly different from those of the controls but were significantly higher than those of the group receiving immediate posttraining injections of 0.05 μg MK-801 ($U=9$, $p<0.05$).

Experiment IV: Lack of Effects of Pretest Intra-Amygdala Injections of AP5 on Retention.

Retention performance is shown in Figure 4. Pretest intra-amygdala injections of AP5 had no significant effect on retention performance at any dose ($H(3)=0.647$, $p<0.50$).

Experiment V: NMDA or NE Attenuated the AP5-Induced Amnestic Effect.

The retention performance is shown in Figure 5. In replicating previous results, pretraining intra-amygdala infusion of 1.25 μg or 5.0 μg AP5 impaired retention. The retention deficit induced by 1.25 μg AP5 was completely abolished by posttraining

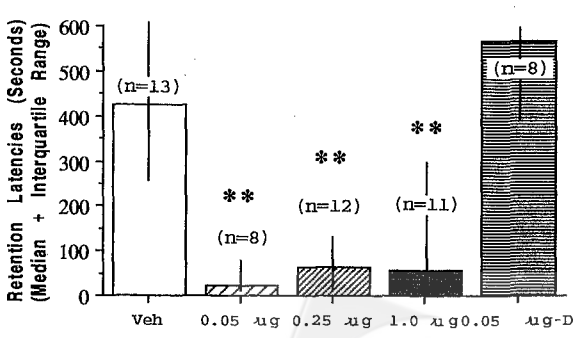

Fig. 3. Effects of posttraining intra-amygdala injection of MK-801 on retention performance. **$p<0.01$ different from the Veh group.

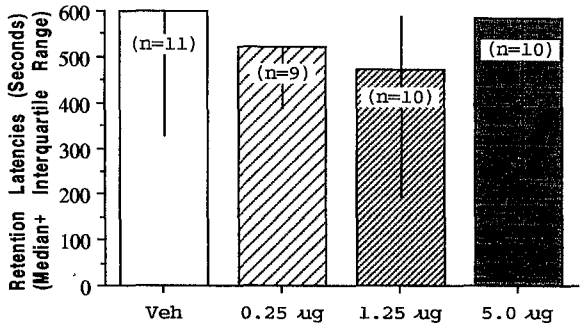

Fig. 4. Lack of effect of pretest intra-amygdala injections of AP5 on retention performance.

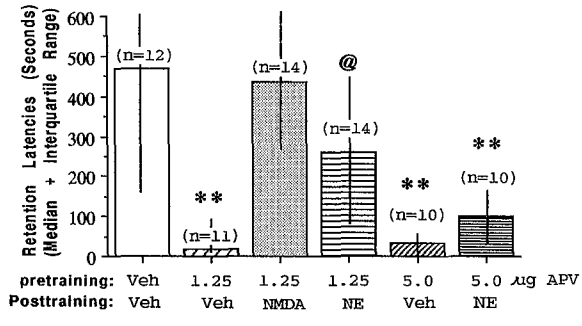

Fig. 5. Attenuation of the AP5-induced amnestic effect by post-training intra-amygdala injections of NMDA or NE. **p <0.01 different from the Veh group, @p <0.05 different from the 1.25 μg AP5/Veh group.

intra-amygdala infusion of 0.25 μg NMDA. Posttraining intra-amygdala infusion of 0.20 μg NE partially attenuated the amnesia induced by 1.25 μg AP5, but barely attenuated that caused by 5.0 μg AP5. A Kruskal-Wallis one-way analysis of variance revealed a significant difference among various groups ($H(5) = 31.82$, $p<0.001$). Multiple paired comparisons by Mann-Whitney U-tests indicated that both the 1.25 μg AP5/Veh group and the 5.0 μg AP5/Veh group had significantly lower retention scores than the Veh/Veh group ($U = 16$ and 21, respectively; $p<0.01$). The 1.25 μg AP5/NMDA group had retention scores significantly higher than the 1.25 μg AP5/Veh group ($U = 5$, $p<0.001$) but not different from the Veh/Veh group, indicating a complete attenuation. Retention performance in the 1.25 μg AP5/NE group was also better than that in the 1.25 μg AP5/Veh group ($U = 14$, $p<0.001$) but seemed to be lower than that in the Veh/Veh group, although the difference failed to reach significance. To increase the power of the test, the Veh/Veh group and the APV/NMDA group were collapsed into a non-amnestic group. The 1.25 μg AP5/NE group had significantly lower retention scores than the non-amnestic group ($U = 127$, $p<0.05$, one-tailed test). The retention scores of the 5.0 μg AP5/NE group were also higher than those of the 5.0 μg AP5/Veh group, but the difference only approached statistical significance ($U = 28$, $0.05<p<0.10$, one-tailed test).

Histology

The injection needle tips were distributed substantially within the amygdala. However, the lateral, basolateral and basomedial nuclei had high densities of needle tips. A photomicrograph of cannula tracts in the amygdala from a representative animal is shown in Fig. 6.

Discussion

The major findings of the present study can be recapitulated as follows: In a 21-day retention test of an inhibitory avoidance response, pretraining intra-amygdala injections of AP5 impaired memory. Post-training intra-amygdala injection of AP5 or MK-801 also caused a memory deficit, and the latter effect was time-dependent. On the contrary, pretest intra-amygdala injection of AP5 did not affect retention performance. Finally, the amnestic effect of pretraining injected 1.25 μg AP5 could be completely abolished by NMDA and at least partially attenuated by NE infused into the same region immediately after training. These results are consistent with previous ones that amygdaloid NMDA receptors are involved in formation, but not expression, of emotional memory (7,25,32,36). Moreover, they showed for the first time that blocking amygdala NMDA receptors around training had a persistent and profound effect on

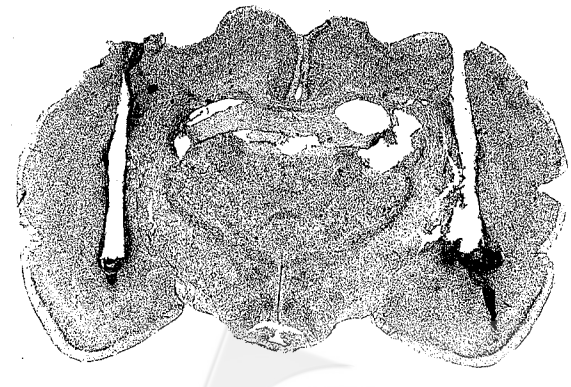

Fig. 6. A photomicrograph of amygdala cannula tracts in a representative animal.

memory tested long after training. Such findings provide clear evidence supporting that NMDA receptors are indeed involved in processing of the enduring affective memory.

The influences of NMDA antagonists on sensorimotor functions or anxiogenesis have led to a suggestion that these drugs may affect performance factors rather than memory processes per se (29). However, several lines of evidence argue against such an interpretation. First, pretest intra-amygdala injections of AP5 had no effect on retention performance, indicating no compromise in capability of light-dark discrimination or fear motivation. Second, the time-dependent effect of posttraining injected MK-801, along with the time-dependent effect of posttraining injected AP5 found in a previous study (36), argues strongly that the drug affected a memory consolidation processes rather than performance factors during acquisition or retrieval (43). In addition, several studies have shown that intra-amygdala injections of AP5 did not affect shock sensitivity or locomotor activity (32,36,47).

The findings that 0.25 μg NMDA abolished the retention-impairing effect of 1.25 μg AP5 rules out that the AP5 effect could have been due to any not-yet demonstrated actions of the drug unrelated to NMDA receptors, a possibility being ignored by most of previous studies. The lack of an AP5 effect in rats treated with NMDA is not likely due to an algebraic summation of two counteractive effects irrelevant in mechanism (42), since 0.25 μg NMDA by itself did not improve retention according to one of our previous studies (36). The fact that a higher dose of NMDA may cause excitotoxic damage on the amygdala prevents the attempt to examine whether the amnestic effect of 5.0 μg AP5 could be blocked by a higher dose of NMDA.

There is consensus on the pronounced memory defects produced by NMDA antagonists given before training, but the effect of these drugs given after training is more controversial. Several studies reported no effect of posttraining injections of NMDA antagonists on acquisition or retention (11,30,59,60), while others showed a clear effect (16,21). The inconsistency may be due to differential drug distributions, in time and concentration, to the target brain regions involved in particular tasks, as all these studies employed systemic or icv injections. By infusing the drug directly into the critical brain regions, this and several other studies (25, 36) demonstrated profound memory effects of posttraining administered NMDA antagonists. It remains unresolved that posttraining intra-amygdala injections of AP5 failed to affect the conditioned fear-potentiated startle task (Davis, personal communication). It should be noted that Davis adopted a multiple-trial training paradigm (at least 5 to 10 trials). Therefore, at the time when the treatment is given, the animal has gone through much learning, which may presumably generate a memory trace mcuh more resistant to posttraining modification. To resolve this discrepancy, it is imperative to examine the effect of immediate posttraining AP5 injections in a one-trial conditioned fear-potentiation of startle paradigm.

While both pretraining and posttraining intra-amygdala injections of AP5 impaired retention, magnitudes of the two effects were by no means identical. The difference was especially apparent at low doses. AP5 at 0.25 μg caused substantial memory deficits when injected before training, but produced no discernible effect if injected after training. For rats receiving 1.25 μg AP5, the pretraining treatment also produced a more apparent deficit than the posttraining treatment. Such findings indicate that blocking amygdaloid NMDA receptors at the moment of acquisition did influence memory processing, although the effect was not immediately apparent as shown by two previous studies indicating lack of effect of pretraining AP5 on acquisition or immediate retention (31,32). Therefore, activation of amygdala NMDA receptors, during and after acquisition, has additive impacts on formation of a long-lasting emotional memory.

An entertaining issue is whether the memory effect of AP5 is related to LTP. An early study suggested that in amygdala slices LTP induced by stimulating the external capsule was not NMDA-dependent (8). However, a recent report showed that AP5 blocked LTP in the basolateral amygdala induced by stimulation of endopiriform nuclei (19). Thus, the marked retention deficits induced by pretraining amygdala-injected AP5 could be taken as supporting evidence for the involvement of amygdala LTP in subserving affective memory (12). In the present study, cannula tips were located mainly around the basolateral amygdala complex with some distribution in other portion of the amygdala such as the central nucleus. Yet amygdala LTP has only been shown in the lateral and basolateral nuclei (8,19). Previous studies have shown that treatments applied to either the basolateral or the central nuclei could affect acquisition/retention performance (3,12). Such results have led to a suggestion that converging sensory stimuli during training forge plastic changes at the lateral and basolateral nuclei, and the central nucleus by receiving inputs from the former two is responsible for integrating amygdala outputs (12,55). The contributions from blocking plasticity and interfering with the output to the effect oboserved remain to be tease apart in the future.

That posttraining AP5 also impaired memory appears to be inconsistent with the findings that AP5

applied after the tetanic stimulation did not block LTP (28), if LTP subserving memory could only be established by neural activities at the time of, but not after, training. However, memory traces in the brain may be forged by reverberating neural activities being set off by, but out-lasting, the sensory stimuli in a learning situation (22). Such a notion was supported by extensive evidence showing that various posttraining manipulations of neural function affect retention performance (45). Stimuli significant to an animal could activate in its amygdala neuronal responses persisting long after the disappearance of the stimuli (50). Longer stimulation provided by multiple tetanus trains would result in more persistent LTP (14). Therefore, AP5 applied immediately after a training experience may block the activation of amygdaloid NMDA receptors, and hence the LTP, induced by neural activities continuing after training and critical for establishing a durable trace. It remains a puzzle for the LTP interpretation that while the NMDA component of EPSP was significantly potentiated in amygdala LTP (19), yet pretest AP5 failed to affect memory expression. The potentiated NMDA component may be involved in other types of amygdala plasticities, such as epileptogensis. Alternatively, the long-term neural trace of affective memory may become independent of the amygdala as suggested by several studies (35, 36).

The present study evaluated the possibility whether AP5 injected into the amygdala may affect long-term retention by altering amygdala noradrenergic functions. Intra-amygdala infusion of 0.2 μg NE, a dose most effective in improving poor retention under low footshock conditions (38,39), could only partially normalize retention in rats given 1.25 μg AP5. The attenuative effect of NE became less apparent as the dose of AP5 was raised to 5.0 μg. Because the attenuation was incomplete even at a memory-enhancing dose of NE, the present findings failed to provide a strong support for that AP5 induces amnesia *exclusively* by blocking the release of NE. As a matter of fact, there is evidence that in the hippocampus, NMDA receptors mediated the NE-enhancing effect on retention of the inhibitory avoidance response (34). However, it remains possible that under certain circumstances, activating amygdaloid NMDA receptors results in two consequences, i.e. inducing LTP and releasing NE, which act cooperatively to produce a long-lasting memory trace. Activating β noradrenergic receptors has been shown to prolong LTP duration in hippocampal slices receiving tetanus stimulation (23). Based on this notion, replacing NE into the amygdala would be a remedy for AP5 only when there is still residual activation of NMDA receptors. While rats receiving 1.25 μg and 5.0 μg AP5 were indistinguishably amnestic in a 21-day retention test, the former group did showed trace of retention in a 1-day test but the latter showed none according to our previous findings (36). It is thus likely that NE amplified and prolonged a weak trace in the 1.25 μg-treated group, which would have otherwise faded within a 21-day period. Such results are consistent with the findings that isoproterenol induced a sustained enhancement of NMDA-dependent evoked EPSP in the amygdala (20), and with the notion that activation of NMDA receptors may instigate multiple processes involved in memory formation.

How amygdala NMDA receptors are activated during learning and how this activation participates memory processing can only be speculated. Excitatory amino acid pathways have been found to project from association cortices to the amygdala (3, 5). These fibers probably transmit well-processed modality-specific or multi-modality sensory information to the amygdala (24), as previous evidence showing that complex sensory stimuli evoked neurophysiological responses in the amygdala (49,54). Lesions of these cortico-amygdala pathways produced marked impairments in learning and memory performance in monkeys or rats (17). It is thus likely that during the inhibitory avoidance training, the electric shock as well as other modalities of sensory stimulation activate recurrent cortical inputs to the amygdala and contribute to the processes of acquiring and retaining new information. As for whether glutamate is indeed released by learning experience is now under investigation.

Acknowledgements

This study was supported by a grant NSC-80-0412-B002-148 from the National Science Council of Republic of China and funds from Department of Psychology, National Taiwan University.

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