Formation and Retrieval of Inhibitory Avoidance Memory: Differential Roles of Glutamate Receptors in the Amygdala and Medial Prefrontal Cortex

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Abstract

This study investigated the roles of NMDA and AMPA receptors in the amygdala and medial prefrontal cortex in formation and retrieval of affective memory. In a one-trial step-through inhibitory avoidance task, groups of rats with cannulae implanted into these two regions received infusion of 2.5 μg APV or 0.3 μg CNQX 5 min before training, shortly after training or 5 min prior to the 1-day or 21-day retention test. Results showed that pre- or posttraining intra-amygdala infusion of APV or CNQX induced a persistent retention deficit with the pretraining treatment causing a greater effect. Pre- or posttraining infusion of CNQX into the medial prefrontal cortex also induced a persistent retention deficit with the posttraining treatment causing a greater effect. Pre- or posttraining infusion of APV into the medial prefrontal cortex impaired 21-day retention but not 1-day retention. Pretraining infusion of lidocaine into either structure caused a retention deficit, which was not attenuated by activating the other structure with glutamate. Pretest intra-amygdala infusion of CNQX impaired memory expression in the 1-day test, while infusion of CNQX into the medial prefrontal cortex impaired memory expression in the 21-day test. Pretest APV infusion into either structure had no effect on memory expression. These findings suggest that the amygdala and medial prefrontal cortex may be contained in a circuitry responsible for formation of affective memory. The consolidation process involves the NMDA and AMPA receptors in both structures. Further, retrieval of recent affective memory engages amygdala AMPA receptors, whereas retrieval of remote affective memory engages AMPA receptors in the medial prefrontal cortex.

Key Words: amygdala, medial prefrontal cortex, NMDA receptors, AMPA receptors, affective memory, rats

Introduction

Emotional experience arouses the organism and leaves a long-lasting memory trace, which is easily reactivated and underlies normal and abnormal mental processes, such as flashbulb memory (14) and post traumatic stress disorders (72). Extensive evidence has implicated the amygdala in memory processing of affective events (1). Various treatments applied to the amygdala during learning affect memory in a time-dependent manner: The closer in time the treatment to training, the greater the subsequent effect on retention (63). These results suggest that the amygdala is involved in formation of affective memory.

However, whether the amygdala stores affective memory is controversial. Some investigators have proposed that the plasticity subserving long-term affective memory resides in the amygdala (51). Others suggest that the amygdala simply exerts modulatory influences on memory without actually storing the acquired information (63). The findings that lesions of the hippocampus or amygdala impaired recently formed memory, but spared memory formed at a

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remote past (52, 84) raise a further possibility that these limbic structures may play an essential role at the initial phase of memory processing, yet are not the permanent storage sites of memory (78).

In view of this debate, an important task is to explore the neural circuitry underlying long-term storage of information. The cerebral cortex has recently been conjectured to subserve such a function (15, 80). In rats, pre- or posttraining inactivation of the insular cortex by tetrodotoxin impaired memory in an inhibitory avoidance task and the Morris water maze task (6). Further, ibotenate lesions of the insular cortex disrupted acquisition and retention of conditioned taste aversion (20). In addition, pretraining electrolytic lesions of the perirhinal cortex blocked conditioned fear-potentiated startle (74). However, most of these studies administered irreversible lesions prior to the training experience. Thus, unambiguous dissociation among the roles of these cortical regions in acquisition, storage and retrieval processes is very difficult, if not impossible.

The amygdala is intimately interconnected with the cerebral cortex (48). It provides the major limbic inputs to the prefrontal cortex including its medial region. The basolateral amygdaloid nucleus projects to the prelimbic and infralimbic prefrontal areas either directly (47, 62), or indirectly by way of the mediodorsal thalamic nuclei (61) or nucleus accumbens and its related structures (18, 77). The prelimbic and infralimbic cortices innervate the amygdala reciprocally (10). Stimulating the basolateral amygdaloid nucleus induces excitatory or inhibitory postsynaptic potentials in the medial prefrontal neurons (71). Previous evidence has suggested that glutamate is the transmitter in some of the cortico-amygdaloid and amygdaloid-cortical fibers (2, 24, 83).

The prefrontal cortex is implicated in learning and memory processes (75). In monkeys, the dorsolateral prefrontal cortex is involved in working memory (29) dealing with spatial (69) or temporal information (25). In rats, disrupting the function of various prefrontal regions also impaired performance in spatial tasks assessing working memory (30, 64, 66, 76, 79, 82), although the exact nature of the deficit is under debate (8, 17). Most of these tasks requested the subjects to hold information for only a short period of time. Therefore, such results do not really bear upon whether the prefrontal cortex stores learned information on a long-term basis.

The medial prefrontal cortex of rats is phylogenetically related to the orbitofrontal region of monkeys (73), which is more involved in affective functions (4). This region is intimately connected with the amygdala both anatomically and functionally. For example, psychological stress increased serotonin release in both the amygdala and prefrontal cortex of rats (44). Lesions of the amygdala abolished prefrontal dopamine turnover under stress (16). The medial prefrontal cortex thus may be involved in memory for positive or negative emotional experience. Indeed, extensive evidence suggests a role of this region in classical or operant conditioned behavior rewarded by brain self-stimulation or drug self-administration (9, 37, 81). Previous studies have shown that pretraining lesions of this area hindered active avoidance acquisition (59), while posttraining stimulation of it by massive currents impaired memory in an inhibitory avoidance task (28).

Long-term potentiation (LTP) is a prevailing neurobiological model for learning and memory (58, 60). In the amygdala, LTP has been elicited in vivo (12) or in vitro (11, 27). In the prefrontal cortex, LTP could be induced in the prelimbic region of an anesthetized rat by stimulating the CA1/subicular area of the hippocampus (19, 50), or elicited in the layer V pyramidal cells of a prefrontal cortical slice by stimulating in layer II fibers (33). Both N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxyl-5-methyl-4-isoxazole propionate (AMPA) receptors are present in the amygdala and prefrontal cortex (31, 68). Evidence has demonstrated NMDA-dependent forms of LTP in both regions (27, 32, 33).

Consistently, previous studies have shown that blocking the amygdaloid NMDA receptors with 2-amino-5-phosphonovaleric acid (APV) shortly before or after training impaired memory in the inhibitory avoidance task (53, 56). Other studies have also shown that intra-amygdala infusion of an AMPA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) impaired acquisition or memory expression in inhibitory avoidance, novelty habituation (38) and conditioned fear-potentiated startle (46), although interpretations for such effects remain somewhat controversial (65). AMPA receptors in the entorhinal cortex have been implicated in expression, but not formation, of memory (40, 42), yet little is known for the roles of NMDA and AMPA receptors in the medial prefrontal cortex.

A previous study has shown that NMDA and AMPA receptors in the amygdala, hippocampus and entorhinal cortex are engaged at different temporal domains during the consolidation phase (39). In addition, our previous findings indicated that the amygdala is critical for expression of inhibitory avoidance memory formed recently (1 day) but not critical for expression of memory formed long time (12 days) ago (52, 54). It would be of interests to know whether the medial prefrontal cortex is also differentially involved in memory consolidation and retrieval of recent or remote memory, which is pertinent to the question of where the long term

storage may reside. To address these issues, experiments were designed to compare the effects on retention induced by pretraining, posttraining or pretest infusions of NMDA or AMPA blockers into each of these two regions.

In primates, memory for rewards has been shown to involve connections between the amygdala and medial prefrontal cortex (26). If these two structures also participate in formation of inhibitory avoidance memory, it would be interesting to learn how they may interact during the consolidation process. To address this question, we examined whether the deleterious effect induced by suppressing one of the two structures with 4% lidocaine (67) could be corrected by activating the other structure with a memory-enhancing dose of glutamate (23, 39), based on the assumption that if processing of these neural signals is in series, input deficiencies might be overcome by directly activating its target.

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 at the animal room 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 amygdala and/or medial prefrontal cortex. They were anesthetized with 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. After being shaved on the head, the anesthetized animal was mounted on a DKI-900 stereotaxic instrument. To implant cannulae into the brain, the incisor bar was set at - 3.3 mm. The coordinates for the amygdala were AP. - 3.0 mm, ML. ± 4.8 mm and DV. - 6.5 mm. Those for the medial prefrontal cortex were A.P. + 3.0 mm, M.L. ± 1.0 mm and D.V. - 3.0 mm. All measurements were based on the skull surface of the bregma. Cannulae were made of 23 G stainless steel tubing with 0.33 mm inner diameter and 0.63 mm outer diameter. The length of cannulae was 15 mm for the amygdala and 10 mm for the medial prefrontal cortex. 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. An intra-muscular injection of antibiotics (bicillin, 40,000 I.U.) was given at the end of surgery. Rats were kept warm until resurrection from the anesthesia. They recuperated for two weeks before any behavioral experiments.

Behavioral Task

The inhibitory avoidance apparatus was a trough-shape alley divided by a sliding door into a safe compartment and a shock compartment, as described elsewhere (52). The safe compartment was lit by a 20 W light bulb and the shock compartment was dark. The rat was placed into the lit site facing away from the door. As the rat turned around, the door was opened. After the rat stepped to the middle of the dark compartment, the door was closed and an inescapable footshock (1.75 mA/1.1 s) was administered through a constant current shocker connected to a timer (Lafayette Instruments, Model 80240 and Model 58010, Lafayette, IN). The shock intensity was calculated as the root mean square of sinusoidal alternating currents.

After shock administration, the rat was removed from the alley and returned to his home cage. In the retention test given 24 hrs later, the rat was reintroduced into the alley and its latency to step into the shock compartment was taken as a retention score. If the rat did not step through in 10 min, the test trial was terminated and a ceiling score of 600 (sec) was assigned.

Drugs and Drug Administration

Lidocaine and DL-APV were obtained from Sigma (St. Louis, MO, U.S.A.), L-glutamic acid hydrochloride was obtained from RBI (Natick, MA, U.S.A.) and CNQX obtained from Tocris (Britol, U.K.) was a generous gift from Dr. Davis of Yale University. The vehicle for lidocaine, APV and glutamate was 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 0.2 M $NaH_2PO_4 \cdot 2H_2O$. The vehicle for CNQX was 1% DMSO.

The intra-cerebral 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 solution was 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 after removing the stylet. To facilitate diffusion of drugs, the infusion 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 intra-cerebral infusion was administered through a microinfusion pump (CMA/100, Carnegie Medicin, Stockholm) at a rate of 0.5 µl per minute. The infusion volume in each side was 1.0 µl for the amygdala and 0.5 µl for the medial prefrontal cortex. After infusion, the needle stayed in the cannula for an additional minute before withdrawn and the stylet was replaced immediately to prevent back flow.

Procedure

Rats bearing chronic cannulae in the amygdala or medial prefrontal cortex were trained on the inhibitory avoidance task. They received one of the following treatments: buffer, 2.5 µg APV, DMSO or 0.3 µg CNQX infused into the implanted area. The treatment was given at one of the following times: 5 min before training, immediately after training or 5 min prior to the retention test. For each treated group, half of the subjects were tested 1 day after training, while the other half were tested 21 days after training. The doses of APV and CNQX were within the range of effective doses according to our previous findings (52, 53). In one experiment, vehicle or 4% lidocaine was administered into one of the two structures 5 min before training. Immediately after training, glutamate or vehicle was infused into the remaining structure. The doses of glutamate were 5.0 µg for the amygdala and 2.5 µg for the medial prefrontal cortex, which were based on findings from a previous study (39). These rats were tested only at the 1-day test.

Histological 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. They were later sectioned into 40 µm slices and stained with cresyl violet. Placements of the cannulae were examined by projecting the stained slides onto a brain atlas chart (70) and recording the location of cannula tips on the chart.

Results

Intra-amygdala infusion of APV before or after training

For rats receiving buffer or DMSO, no statistical significant difference in retention scores was found between the pretraining and posttraining infusion paradigms in this and all other experiments, thus in each experiment they were collapsed into a pooled control group. The effects of pretraining or posttraining intra-amygdala infusion of 2.5 µg APV on 1-day and 21-day retention are shown in Figure 1. Because the score distribution was truncated at 600, medians and interquartile ranges are used to represent the central and dispersion tendencies, respectively. Nonparametric statistics were applied to test the effect.

In replicating previous results, APV infused into the amygdala shortly before or after training impaired memory in the 1-day test and this APV-induced deficit persisted up to 21 days after training. In the 1-day test, a Kruskal-Wallis one-way ANOVA revealed significant differences among the groups ($H(2) = 13.6$, $p < 0.005$). Paired comparisons by Mann-Whitney two-tail U-tests revealed that the group receiving either pretraining or posttraining APV infusion had poorer retention than the pooled Buf group ($U = 36$ and 31, for pretraining and posttraining infusions, respectively; $p < 0.005$). While rats receiving pretraining APV infusion appeared to have poorer retention than those receiving posttraining infusion, the difference failed to reach statistical significance ($U = 26$, $p > 0.10$).

In the 21-day test, a Kruskal-Wallis one-way ANOVA revealed significant differences among the groups ($H(2) = 18.8$, $p < 0.001$). Rats infused with APV before or after training had poorer retention than the pooled Buf group ($U = 12$ and 44.5, for pretraining and posttraining infusions, respectively; $p < 0.001$). The pretraining APV infusion group had significantly lower retention scores than the posttraining APV infusion group ($U = 15$, $p < 0.05$). Retention scores in the pooled Buf groups showed no significant

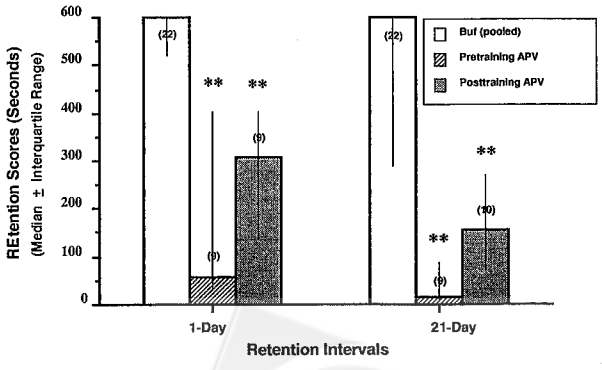

Fig 1. Effects of pre- or posttraining intra-amygdala infusion of 2.5 µg APV on 1-day and 21-day retention. Performance is indicated by the median and the interquartile range of each group. Numbers in the parentheses denote the sample size in all figures. ** $p < 0.01$ different from the correspondent pooled Buffer group.

difference between the 1-day and the 21-day tests. APV-treated rats appeared to have deteriorating retention within a 20-day interval, however, only the difference in the pretraining groups approached statistical significance ($U = 19$, $p < 0.06$).

Intra-Amygdala Infusion of CNQX before or after Training

Posttraining intra-amygdala infusion of DMSO appeared to have a slight memory impairing effect in comparison with the buffer controls of Experiment I ($U = 169$, $z = 2.7$, $p < 0.01$ and $U = 175$, $z = 2.2$, $p < 0.05$ for 1- and 21-day retention, respectively). The effects of pretraining or posttraining intra-amygdala infusion of 0.3 µg CNQX on 1-day and 21-day retention are shown in Figure 2. CNQX infused into the region shortly before or after training induced long-lasting retention deficits. In the 1-day test, a Kruskal-Wallis one-way ANOVA revealed significant differences among the groups ($H(2) = 19.7$, $p < 0.001$). Paired comparisons revealed that rats receiving either pretraining or posttraining CNQX infusion had poorer retention than the pooled DMSO group ($U = 35.5$, $p < 0.001$ and $U = 102.5$, $p < 0.05$ for pretraining and posttraining infusions, respectively). Rats receiving pretraining CNQX infusion had significantly poorer retention than those receiving posttraining CNQX infusion ($U = 27.5$, $p < 0.01$).

In the 21-day test, a Kruskal-Wallis one-way ANOVA revealed a significant difference among the groups ($H(2) = 19.2$, $p < 0.001$). Rats infused with CNQX before or after training had lower retention scores than the pooled DMSO group ($U = 32$, $p < 0.001$ and $U = 77$, $p < 0.01$ for pretraining and posttraining infusions, respectively). The pretraining CNQX infusion group had significantly poorer retention than the posttraining CNQX infusion group ($U = .22$, $p < 0.005$). The pooled DMSO groups did not differ in retention scores between the 1-day test and the 21-day test ($U = 268$, $z = 1.3$, $p > 0.10$). For rats receiving CNQX into the amygdala, memory over the 20-day retention period deteriorated significantly in the posttraining group ($U = 45.5$, $p < 0.05$). The deterioration in pretraining CNQX-treated groups was only marginally significant ($U = 49.5$, $p < 0.075$), which was probably due to a floor effect because retention scores of pretraining CNQX rats in the 1-day retention test were already very low.

Infusion of APV into the Medial Prefrontal Cortex before or after Training

Retention scores for various groups are shown in Figure 3. In contrast to being infused into the amygdala, APV infused into the medial prefrontal cortex before or after training did not affect retention in the 1-day test: A Kruskal-Wallis one-way ANOVA did not reveal a statistical significance among various groups ($H(2) = 1.5$, $p > 0.1$). The group receiving posttraining infusion of APV appeared to have poorer retention than the correspondent Buf-treated controls, but the difference was not statistically significant ($U = 49$, $p > 0.10$). Further, no difference was found between the pretraining treated group and the posttraining treated group ($U = 30$, $p > 0.10$).

However, the same treatments produced marked impairing effects in the 21-day test: A Kruskal-Wallis one-way ANOVA revealed a significant difference among groups ($H(2) = 15.7$, $p < 0.001$). Further paired comparisons indicated that pre- or posttraining APV infusion into the medial prefrontal cortex induced a pronounced 21-day retention deficit: Both APV-

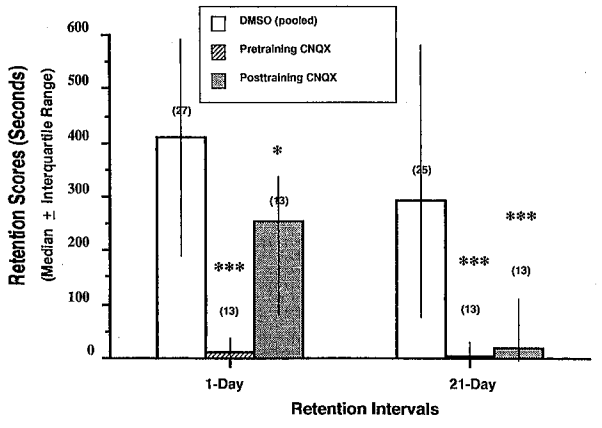

Fig 2. Effects of pre- or posttraining intra-amygdala infusion of 0.3 µg CNQX on 1-day and 21-day retention. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ different from the correspondent pooled DMSO group.

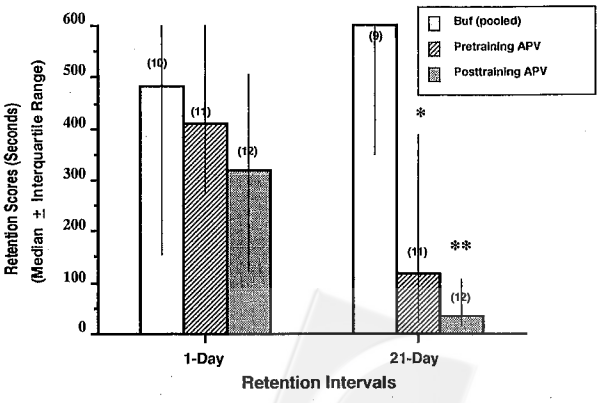

Fig 3. Effects of pre- or posttraining infusion of 2.5 µg APV into the medial prefrontal cortex on 1-day and 21-day retention. * $p < 0.05$; ** $p < 0.01$ different from the correspondent pooled Buffer group.

treated groups had significantly poorer retention than the pooled Buf group ($U = 20$, $p < 0.05$ and $U = 10.5$, $p < 0.01$ for pretraining and posttraining infusions, respectively). No difference was found between the effects produced by the pre- and posttraining treatments. Further, while there was no significant difference in retention between the Buf groups in the 1-day and 21-day tests, the 21-day retention was significantly lower than the 1-day retention in the pre- or posttraining APV infusion groups ($U = 26$, $p < 0.05$ and $U = 20$, $p < 0.01$, respectively).

Infusion of CNQX into the Medial Prefrontal Cortex before or after Training

Retention scores for various groups receiving infusion of DMSO or CNQX into the medial prefrontal cortex are shown in Figure 4. Pre- or posttraining infusion of CNQX into the medial prefrontal cortex induced a retention deficit with the posttraining treatment causing a greater effect. In the 1-day test, a Kruskal-Wallis one-way ANOVA revealed a significant difference among the groups ($H(2) = 15.7$, $p < 0.001$). While rats receiving pretraining CNQX infusion appeared to have lower retention scores than the controls, the difference fell short of statistical significance ($U = 28$, $p = 0.1$). On the other hand, rats receiving posttraining CNQX infusion had significantly poorer retention than the controls ($U = 2$, $p < 0.001$) and rats receiving pretraining CNQX infusion ($U = 6$, $p < 0.01$).

In the 21-day test, a Kruskal-Wallis one-way ANOVA revealed a significant difference among various groups ($H(2) = 13$, $p < 0.002$). Further paired comparisons indicated that the controls had better retention than rats receiving pretraining or posttraining CNQX ($U = 19$, $p < 0.05$ and $U = 5$, $p < 0.001$; respectively), while the latter two groups did not differ from each other. No difference was found in retention scores between the 1-day and the 21-day tests for all correspondent groups.

Interaction between the Amygdala and Medial Prefrontal Cortex during Memory Formation

This experiment examined whether the amnesic effect induced by pretraining lidocaine (Lid) inactivation of the amygdala or medial prefrontal cortex could be reversed by posttraining glutamate (Glu) stimulation of the other structure. Five groups having various amygdala/medial prefrontal cortex treatments are abbreviated as the Veh/Veh, Lid/Veh, Veh/Lid, Lid/Glu and Glu/Lid groups. Retention scores are shown in Figure 5. Pretraining infusion of 4% lidocaine into either structure caused marked retention deficits. However, the deficit such elicited was not attenuated by glutamate stimulation of the otherwise untreated structure. A Kruskal-Wallis one-way ANOVA revealed a significant difference among the groups ($H(4) = 29.8$, $p < 0.001$). Paired comparisons revealed that rats receiving lidocaine in the amygdala before training had poorer retention than the controls (Lid/Veh vs. Veh/Veh, $U = 4$, $p < 0.0001$). Posttraining glutamate infusion into the medial prefrontal cortex failed to attenuate this deficit: the Lid/Glu group had scores similar to those of the Lid/Veh group, but significantly lower than those of the Veh/Veh group ($U = 32$, $p < 0.001$). Rats receiving lidocaine in the medial prefrontal cortex before training also had poorer retention than the controls (Veh/Lid vs. Veh/Veh, $U = 9$, $p < 0.001$). Posttraining intra-amygdala infusion of glutamate failed to attenuate this deficit: The Glu/Lid group had scores similar to those of the Veh/Lid group, but significantly lower than those of the Veh/Veh group ($U = 17$, $p < 0.01$).

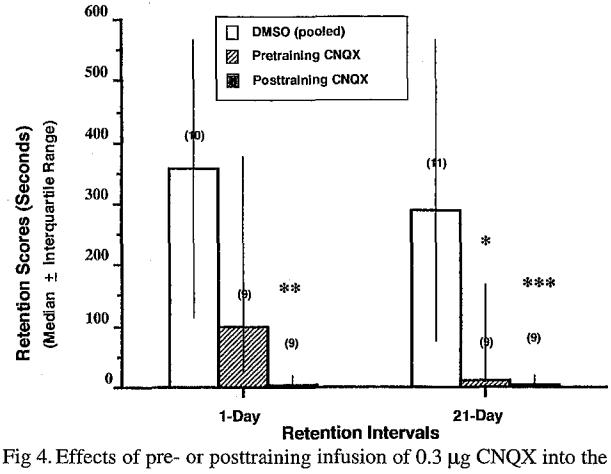

Fig 4. Effects of pre- or posttraining infusion of 0.3 μg CNQX into the medial prefrontal cortex on 1-day and 21-day retention. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ different from the correspondent pooled DMSO group.

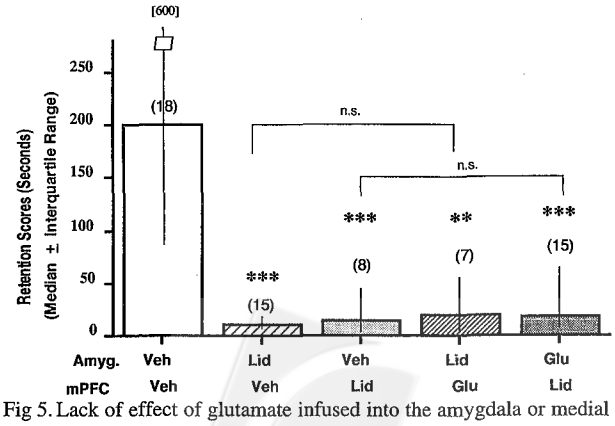

Fig 5. Lack of effect of glutamate infused into the amygdala or medial prefrontal cortex in counteracting the amnesia induced by lidocaine suppression of the other structure. $***p < 0.001$ different from the Veh/Veh group.

Intra-Amygdala Infusion of APV or CNQX before Retention tests

Retention scores for rats receiving pretest intra-amygdala treatments are shown in Figure 6. In replicating previous findings, pretest intra-amygdala infusion of 2.5 μg APV did not affect memory: The APV-treated rats had retention not significantly different from the Veh-treated rats in either 1-day or 21-day retention. In contrast, pretest infusion of 0.3 μg CNQX impaired retention, but only when it was given prior to the 1-day retention test. The CNQX group had significantly lower retention scores than the DMSO group in the 1-day test ($U = 26$, $p < 0.01$), but not in a 21-day retention test. Further, while the Veh groups did not differ in the 1-day and 21-day retention scores, for rats receiving posttraining CNQX infusion, the 1-day retention scores were significantly lower than the 21-day retention scores ($U = 27$, $p < 0.01$).

Infusion of APV or CNQX into the Medial Prefrontal Cortex before Retention Tests

Retention scores for various groups receiving pretest treatments in the medial prefrontal cortex are shown in Figure 7. As found for pretest intra-amygdala infusion of APV, pretest infusion of APV into the medial prefrontal cortex had no effect on either 1-day or 21-day retention performance. Pretest infusion of CNQX into the medial prefrontal cortex suppressed retention in the 21-day test, but had no effect in the 1-day test. For the 1-day test, retention in the CNQX group did not differ from that in the Veh group. Rats given vehicle appeared to show deteriorated memory over the 20-day retention period, but the difference was not statistically significant ($U = 57$, $p > 0.10$). In the 21-day test, retention in the CNQX group was

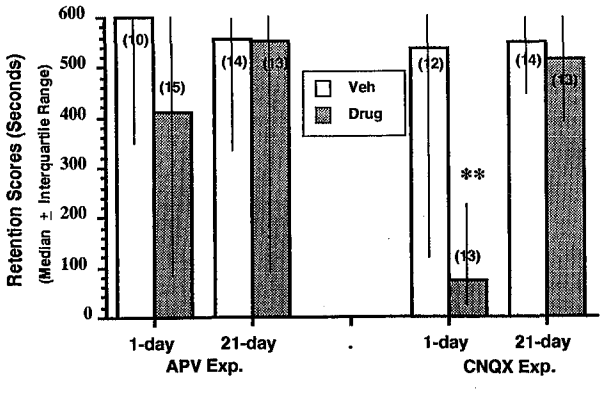

Fig 6. Effects of intra-amygdala infusion of 2.5 μg APV or 0.3 μg CNQX before the 1-day or 21-day retention test on retention performance. ** $p < 0.01$ different from 1-day retention scores of the correspondent Veh group.

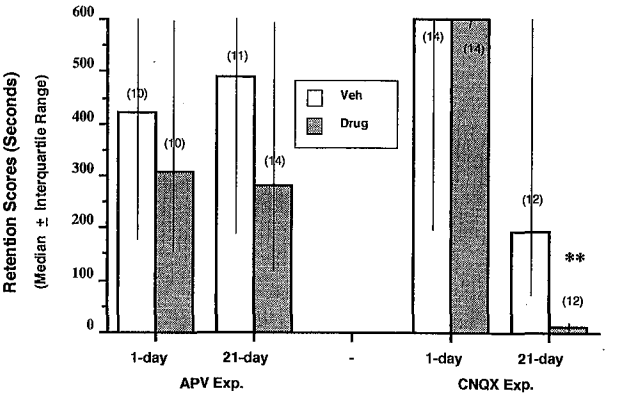

Fig 7. Effects of infusion of 2.5 μg APV or 0.3 μg CNQX into the medial prefrontal cortex before the 1-day or 21-day retention test on retention performance. ** $p < 0.01$ different from 21-day retention scores of the correspondent Veh group.

significantly lower than that in the controls ($U = 13$, $p < 0.001$). Further, for the CNQX-treated animals, those tested 21-days after training had significantly lower scores than those tested 1 day after training ($U = 5$, $p < 0.0001$).

Histology

Cannula tips were mostly located in the basolateral complex of the amygdala and prelimbic or infralimbic regions of the medial prefrontal cortex. Typical cannula tracts in the amygdala and medial prefrontal cortex of representative rats are shown in the upper and lower panels of Figure 8, respectively.

Discussion

Findings from this study could be recapitulated as follows: First, pretraining or posttraining intra-amygdala infusion of APV or CNQX impaired 1- and 21-day retention in the inhibitory avoidance task, but the pretraining treatment caused a greater effect. Second, pretraining or posttraining infusion of APV into the medial prefrontal cortex exerted a deleterious effect on retention which appeared only in the 21-day test. Posttraining infusion of CNQX into the medial prefrontal cortex impaired 1- and 21-day retention, while pretraining CNQX infusion induced less impairments. Third, pretraining infusion of lidocaine into either the amygdala or medial prefrontal cortex caused severe retention deficits, which could not be attenuated by stimulating the other structure with glutamate. Fourth, pretest infusion of CNQX into the amygdala blocked memory expression in the 1-day test, but had no effect on the 21-day test. Conversely, the same treatment applied to the medial prefrontal cortex blocked memory expression in the 21-day test but had no effect in the 1-day test. Pretest infusion of

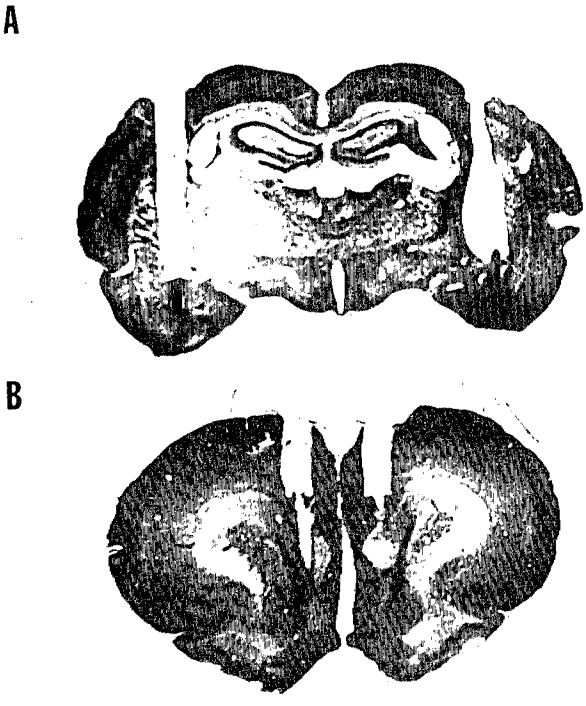

Fig 8. Photomicrographs of typical cannula tracts in the amygdala (upper panel) and medial prefrontal cortex (lower panel) of representative rats.

APV into either region had no effect on retention.

Drugs administered prior to training or testing might affect retention by influencing sensory, motor or motivational factors instead of memory processes per se. However, because pretest infusion of APV into neither structure had any effect on retention, the amnesic effect caused by pretraining infusion of APV is not likely to be due to influences on performance factors, a conclusion also supported by previous findings (9, 38, 53). Central infusion of CNQX reduced shock sensitivity and increased locomotor activity (65), which might account for the greater effect of infusing CNQX into the amygdala prior to training. However, such an interpretation fails to explain the greater effect of CNQX infused into the medial prefrontal cortex after training. The effect of pretest CNQX infusion on test performance is not due to altered locomotor activity or visual discrimination ability, otherwise CNQX should have had the same impairing effects on both 1-day and 21-day tests. Our recent data has shown that posttraining infusion of lidocaine into the amygdala or medial prefrontal cortex resulted in a retention deficit similar to that induced by pretraining infusion (35), thus involvement of performance factors in the lidocaine effect is unlikely.

That pretraining infusion of APV into the cerebral ventricle or some specific neural structures impairs memory is well documented and consistent with the notion that LTP induction in certain brain regions depends upon activation of NMDA receptors (49). Keeping in line with the evidence that APV did not affect established LTP if applied after the tetanic stimulation (13), posttraining administration of NMDA blockers has been justified by some investigators to have no effect on retention (58). The present results, by replicating our previous ones (52), firmly establish that posttraining intra-amygdala APV infusion impairs retention in the inhibitory avoidance task, and support our former conclusion that neural activity may last for a short period of time in the amygdala after termination of the training stimuli and play a role in plasticity underlying memory formation (53).

Two previous studies have shown that posttraining intra-amygdala infusion of CNQX impaired retention of acquired response inhibition or disinhibition (39, 42). Yet another study reported that in conditioned fear-potentiation of startle, pretrial intra-amygdala infusion of CNQX failed to block extinction (21), in which rats have to learn that the original contingency no longer prevails. The present study showed that in the inhibitory avoidance task, either pretraining or posttraining intra-amygdala infusion of CNQX impaired memory. These results are consistent with a view that NMDA-dependent LTP in the amygdala may play an important role in formation of affective memory and amygdala AMPA receptors are crucial for the depolarization required in unblocking the NMDA channel. In the hippocampus, these receptors have also been proposed to have similar roles in acquisition of spatial information (57).

The amnestic effects of APV and CNQX were demonstrated by most of the previous studies in 1-day retention tests. The present results extend these findings by showing that the amnestic effect of either drug was robust at 21 days after training. Thus the observed amnesia could not be due to any suppressing effect of the drug extending from the training period to the retention test. Moreover, by comparing the effects of pretraining and posttraining treatments in the same experiment, the present study reveals that pretraining intra-amygdala infusion of either drug produced a greater amnesic effect than posttraining infusion. Such results imply that the activation of amygdala NMDA and AMPA receptors during training may be more crucial for formation of memory than that after training, although the role of the latter should not be ignored according to our previous discussion.

Complementary to the findings in the amygdala, the present results also show that posttraining infusion of CNQX into the medial prefrontal cortex caused a marked memory deficit in both 1-day and 21-day

tests. The effect of pretraining infusion of CNQX into the medial prefrontal cortex was less clear-cut in the 1-day test. Nonetheless, this subliminal amnesia became apparent as the retention interval lengthened to 21 days. These findings, taken together, suggest that AMPA receptors in the medial prefrontal cortex are involved in formation of inhibitory avoidance memory too. However, these receptors are probably engaged into the consolidation process at a time later than when amygdala AMPA receptors are engaged, because in the medial prefrontal cortex the posttraining CNQX infusion is more debilitating than the pretraining one. CNQX may block the glutamatergic transmission of afferent fibers projecting from other cortical or subcortical areas to the medial prefrontal cortex, and hence impairs memory processing.

Blocking NMDA receptors in the medial prefrontal cortex during training generates an interesting effect. Pretraining or posttraining infusion of APV into this cortical region had no apparent effect on 1-day retention, but both treatments induced a marked deficit if animals were tested 21 days after training. The effectiveness of APV in impairing memory with long retention intervals is consistent with the finding that LTP in the medial prefrontal cortex is NMDA-dependent (34). However, these data can not rule out the possibility that APV in the medial prefrontal cortex impairs memory through mechanisms other than blocking LTP, such as suppressing NMDA-mediated release of other neurotransmitters also involved in learning-related neural plasticity (36), e.g. norepinephrine (43). If the amnesic effect of APV in the medial prefrontal cortex is indeed related to blockade of LTP underlying memory, then the present results could imply that the medial prefrontal cortex and the plasticity therein may be involved in long-term storage of emotional experience.

Glutamate, at a dose of 5.0 μg, has been shown to enhance memory when given immediately after training into either the amygdala (39) or cerebroventricle (23). In the present study, glutamate infused into either the medial prefrontal cortex or amygdala failed to ameliorate the memory deficit due to lidocaine-suppression of the other structure. Such results could suggest that the amygdaloid output to the medial prefrontal cortex and vice versa can not be mimicked by plain glutamate-induced excitation. Alternatively, these data are consistent with a view that the amygdala and medial prefrontal cortex form a circuitry loop for processing affective events. Breakage anywhere in the loop interrupts reverberation, which can not be reinstated by stimulation of any other sites in the loop, and thus disrupts memory consolidation (32).

The present study showed that pretest infusion of CNQX into the amygdala or the medial prefrontal cortex differentially affected retrieval of memory, which is consistent with the notion that expression of LTP depends upon activation of AMPA receptors (49). When infused into the amygdala, CNQX given prior to the retention test suppressed memory expression in the 1-day test. Similar findings have also been demonstrated in the conditioned fear-potentiation of startle task (46). On the other hand, the same treatment applied to the medial prefrontal cortex suppressed memory expression in the 21-day test. Therefore, the amygdala is critical for recollecting a recent (1-day old) affective event, while the medial prefrontal cortex is critical for recollecting a remote (21-day old) one.

Consistent with our findings, Izquierdo and his colleagues (40) have previously shown that pretest infusion of CNQX into the amygdala, hippocampus or entorhinal cortex impaired retrieval of inhibitory avoidance memory in a 1-day test (55). However, they have also shown that while individual blockade of the amygdala or hippocampus during a 20-day retention test had no effect on memory expression, yet simultaneous blockade of the two induced a significant deficit (7). It should be noted that the medial prefrontal cortex receives dense projections from the amygdala (61) and hippocampus (22, 41). It is likely that Izquierdo and his colleagues in fact deactivated the medial prefrontal cortex by depriving two of its major inputs. A similar mechanism has also been proposed in a former model of visual recognition memory in primates (3).

The finding that suppression of the medial prefrontal cortex during testing impaired retrieval is consistent with, but does not prove, that long-term storage of affective events may involve this cortical region. Other interpretations are available. The prefrontal cortex of primate is implicated in working memory (29), a holding buffer for information reactivated from storage. Simply blocking the buffer without disturbing the storage could also prevent long-term memory from expressing. If the medial prefrontal cortex of rats also assumes such a role, it follows that pre- or posttraining suppression of this region impairs memory formation, because rehearsals in working memory are supposed to facilitate registration of new information into the long-term storage. However, this view is incongruent with our results that pretest inactivation of the medial prefrontal cortex impaired memory retrieval in the 21-day test but had no effect in the 1-day tests. Alternatively, expression of inhibitory avoidance memory in the 1-day and 21-day tests could be supported by different behavioral strategies, e.g. passively freezing and actively avoiding, respectively. Therefore, for successful expression of memory at various times

after its formation, distinct retrieval mechanisms may engage different neural structures.

The present results are somewhat different from those based on conditioned freezing behavior or conditioned fear-potentiation of startle. In the latter task, lesions of the amygdala made after an extended training period still abolished conditioned fear (45) which was on the contrary impervious to lesions of the prefrontal cortex (74). It should be noted that in the fear potentiation of startle task, affective memory is indicated by a classical conditioned fear response, while in the inhibitory avoidance task, affective memory is indicated by operant avoidance behavior. It is likely that aversive classical conditioning and aversive operant conditioning engage overlapping but not identical neural substrates in acquisition and storage of affective experience. Further pursue on this track may lead to reconciliation of the discrepancies existing in the presently available results.

On the basis of our findings, affective memory may be speculated to form in a circuitry regulated by the amygdala and then move after a couple of days to another one involving the medial prefrontal cortex. However, such a notion is incongruent with our findings that the medial prefrontal cortex joins in the memory formation process shortly after training. It could be otherwise conjectured that inhibitory avoidance training activates the amygdala and its associated structures. The medial prefrontal cortex is recruited into this circuitry and activity reverberating within which forges consolidation. The activity first sets up an initial neural representation of the experience, which requires an integrated amygdala for its operation and is responsible for the near future recollection. Further, the same activity also initiates some slow plastic changes in a cortical circuitry (5) involving the medial prefrontal cortex to form an additional representation, which matures much later but lasts much longer to support the durable affective memory. This proposal should be evaluated by further research.

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