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Infusions of AP5 into the basolateral amygdala impair the formation, but not the expression, of step-down inhibitory avoidance

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Abstract

We evaluated the effects of infusions of the NMDA receptor antagonist D,L-2-amino-5-phosphonopentanoic acid (AP5) into the basolateral nucleus of the amygdala (BLA) on the formation and expression of memory for inhibitory avoidance. Adult male Wistar rats (215-300 g) were implanted under thionembutal anesthesia (30 mg/kg, *ip*) with 9.0-mm guide cannulae aimed 1.0 mm above the BLA. Bilateral infusions of AP5 (5.0 µg) were given 10 min prior to training, immediately after training, or 10 min prior to testing in a step-down inhibitory avoidance task (0.3 mA footshock, 24-h interval between training and the retention test session). Both pre- and post-training infusions of AP5 blocked retention test performance. When given prior to the test, AP5 did not affect retention. AP5 did not affect training performance, and a control experiment showed that the impairing effects were not due to alterations in footshock sensitivity. The results suggest that NMDA receptor activation in the BLA is involved in the formation, but not the expression, of memory for inhibitory avoidance in rats. However, the results do not necessarily imply that the role of NMDA receptors in the BLA is to mediate long-term storage of fear-motivated memory within the amygdala.

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Key words
- Amygdala
- NMDA receptor
- Fear
- Memory

Introduction

Extensive evidence suggests that the amygdala, particularly the basolateral nucleus (BLA), is involved in emotional memory processing (for reviews, see 1-3). Among other systems, *N*-methyl-D-aspartate (NMDA) glutamate receptors in the amygdala have been implicated in fear-motivated learning (2,4-15). Infusion of the NMDA receptor antagonist D,L-2-amino-5-phosphonopentanoic acid (AP5) into the amygdala blocks acquisition of contextual fear conditioning (5,7,12), second-order fear conditioning (6), conditioned fear-potentiated startle (4,13), step-through inhibitory avoidance (9,11), and step-down inhibitory avoidance (7,8). Although the role of the amygdaloid NMDA receptor in the formation of memory for fear-motivated tasks is well established, its involvement in memory expression remains unclear. Intra-amygdala AP5 blocks both the acquisition and the expression of contextual fear conditioning (12), but does not affect

expression of fear-potentiated startle (3,13) or step-through inhibitory avoidance (11).

It has been postulated that the amnestic effects of intra-amygdala infusions of AP5 are related to a blockade of neural plasticity processes such as NMDA-dependent long-term potentiation (LTP) at amygdaloid glutamatergic synapses (2,5,6,10,13,14). In support of this view, it was recently shown that fear conditioning induces LTP-like changes in auditory-evoked potentials in the lateral nucleus of the amygdala, suggesting a role for LTP in the amygdala in fear memory (14). However, extensive evidence suggests that the amygdala is a modulatory site which regulates memory storage and/or consolidation in other brain regions, rather than a critical site for plasticity underlying memory acquisition and storage (1,3,16-20). This view is supported by studies showing that amygdaloid lesions do not block contextual fear conditioning (20) and retention of inhibitory avoidance (16). Moreover, overtraining can attenuate the amnestic effects of amygdaloid lesions (18), intra-amygdala infusions of NMDA receptor antagonists (9) and intra-amygdala infusions of the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (17,19).

In the present study, we investigated the role of amygdaloid NMDA receptors in the formation and expression of fear memory by evaluating the effects of pretraining, post-training, and pretest infusions of AP5 on retention of a step-down inhibitory avoidance task in rats. A control experiment was carried out in order to evaluate the effects of AP5 on footshock sensitivity.

Material and Methods

Subjects

Eighty-three adult male Wistar rats (215-300 g) obtained from our breeding colony, housed five to a cage with food and water available *ad libitum*, were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). Behavioral tests were conducted between 13:30 and 16:30.

Surgery

Animals were implanted under thionembutal anesthesia (30 mg/kg, *ip*) with 9.0-mm guide cannulae (1.0-mm diameter) aimed 1.0 mm above the BLA. Coordinates relative to bregma, obtained from the atlas of Paxinos and Watson (21) and adjusted according to pilot data, were AP -2.3 mm, ML ±5.0 mm, and DV -6.0 mm from the dura mater.

Behavioral procedures

Inhibitory avoidance. Animals were submitted to the behavioral procedure 3 to 7 days after surgery. The inhibitory avoidance apparatus was a 50 x 25 x 25-cm acrylic box whose floor consisted of parallel stainless steel bars (1 mm in diameter) spaced 1 cm apart. A 7-cm wide, 2.5-cm high platform was placed on the floor of the box against the left wall. Animals were placed on the platform and their latency to step down on the grid with all four paws was measured with an automatic device. In the training session, immediately after stepping down on the grid, the animals received a 0.3 mA, 2.0-s scrambled footshock. In the test session, carried out 24 h after training, no footshock was administered and the step-down latency was used as a measure of retention (7,8).

Footshock sensitivity test. Reactivity to the footshock was evaluated in the same apparatus as used for inhibitory avoidance, except that the platform was removed. The "up and down" method (17,22) was modified as previously described (15,23) and used to determine the nociceptive thresholds. Each animal was placed on the grid and allowed a 2-min habituation period prior to the start of a series of shocks (0.5 s), delivered at 10-s intervals. Shock intensities ranged from 0.1 to 0.5 mA in 0.1 mA increments. The adjust-

ments in shock intensity were made in accordance to each animal's response. The intensity was raised by one unit when no response occurred and lowered by one unit when a response was made. A "flinch" response was defined as withdrawal of one paw from the grid floor, and a jump response was defined as withdrawal of three or four paws. Two measurements of the "flinch" threshold and then two measurements of the "jump" threshold were made. The mean of the two scores for the flinch and the jump thresholds were calculated for each animal (15,17,22,23).

Drugs and infusion procedures

Ten minutes prior to training, immediately after training, or 10 min prior to the retention test session in the inhibitory avoidance task, or 10 min prior to the footshock reactivity test, an infusion cannula was fitted into the guide cannula. The tip of the infusion cannula protruded 1.00 mm beyond the guide cannula and was aimed at the BLA. Through the infusion cannula animals received a bilateral 0.5-µl infusion of vehicle (phosphate buffer in saline, pH 7.4) or AP5 (5.0 µg) (Research Biochemicals International, Natick, MA, USA) dissolved in vehicle (4,5,12). Vehicle or AP5 was infused over a period of 2 min, and the infusion cannulae were left in place for an additional minute to allow diffusion of the drug away from the cannula tip. The dose of AP5 and the volume of infusion were chosen on the basis of previous studies showing that 0.5-µl infusions of 5.0 µg of AP5 are adequate for the study of the effects of AP5 infused specifically into the BLA on memory (4,12).

Histology

Postmortem verification of cannula placement was performed as described in previous papers (7,8,15). Briefly, animals were killed by decapitation and 0.5 µl of a solution of methylene blue in saline was infused through the cannulae. Brains were stored in formalin for at least 72 h and cannula placement was verified by histological examination. Cannulae were found to be correctly placed in the BLA in 73 rats (Figure 1). Data from only these animals were included in the final analysis.

Statistical analysis

Data for inhibitory avoidance are shown as mean ± SEM retention test latencies. Data for the footshock reactivity test are shown as mean ± SEM flinch and jump thresholds expressed as milliamps (mA) (15,23). In all experiments, comparisons between the vehicle and the AP5 groups were performed using the unpaired *t*-tests. In all comparisons, $P<0.05$ was considered to indicate statistical significance.

Results

The first experiment was designed to evaluate the effects of pretraining infusion into the BLA on inhibitory avoidance retention. Results are shown in Figure 2A. The pretraining intra-amygdala infusion of AP5 impaired retention test performance of the inhibitory avoidance task. There was a significant difference between groups in retention test session step-down latency (unpaired *t*-test, $t = 4.00$, $P<0.01$), but not in training session step-down latency (unpaired *t*-test, $t = -0.09$, $P = 0.90$). In addition, AP5 impaired retention test performance when infused

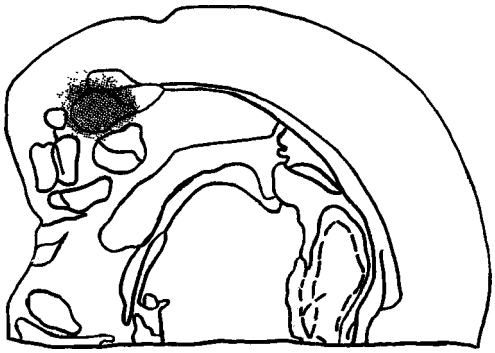

Figure 1 - Drawing of the A -2.3 mm plane of the Paxinos and Watson (21) atlas showing the area (hatched) where the microinjections considered to be correct were placed.

immediately after training (Figure 2B). Again, there was a significant difference between groups in retention test session step-down latency (unpaired *t*-test, t = 4.01, P<0.01), but not in training session step-down latency (unpaired *t*-test, t = -0.09, P = 0.93).

When given prior to the retention test session, AP5 did not affect retention (Figure 2C). There were no significant differences between groups in the training (unpaired *t*-test, t = 1.48, P = 0.16) or in the retention test (unpaired *t*-test, t = 1.16, P = 0.26) performance. The results indicate that the infusion of AP5 into the BLA did not affect the expression of memory for inhibitory avoidance.

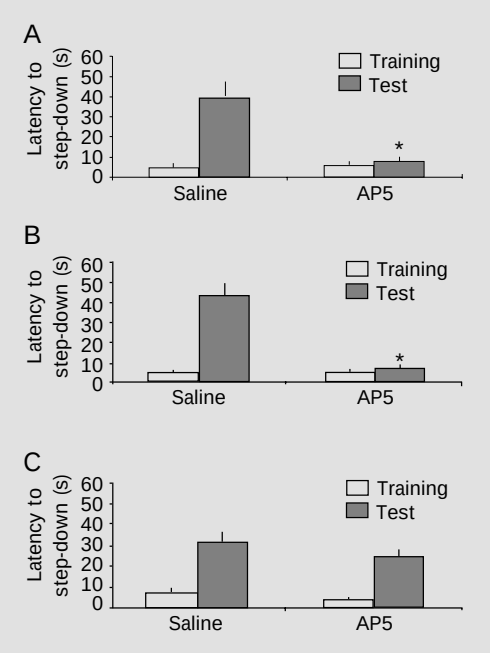

Figure 2 - Effects of pretraining (A), post-training (B), and pretest (C) infusions of D,L-2-amino-5-phosphonopentanoic acid (AP5) (5.0 µg) into the basolateral nucleus of the amygdala on retention of an inhibitory avoidance task. Data are means ± SEM of the step-down latencies; N = 8-11 animals per group. *P<0.01 compared to the vehicle group (unpaired *t*-test).

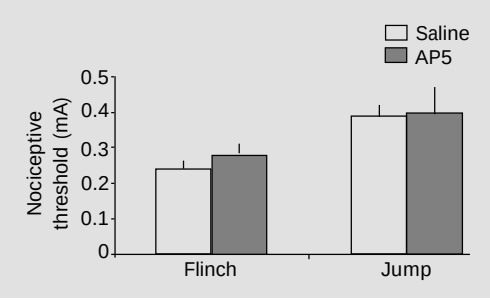

Figure 3 - Effect of intra-amygdala infusion of D,L-2-amino-5-phosphonopentanoic acid (AP5) on reactivity to the footshock. Data are flinch and jump thresholds expressed as milliamps (mA). Animals were given an infusion of AP5 (5.0 µg) into the basolateral nucleus of the amygdala 10 min prior to testing for nociceptive thresholds; N = 8 animals per group. There were no significant differences between groups.

To examine if the deficits in inhibitory avoidance memory were due to an AP5-induced shift in footshock sensitivity, we verified the effects of intra-amygdala AP5 on reactivity to the inhibitory avoidance box footshock, assessed by flinch and jump thresholds. Results are shown in Figure 3. Consistent with previous findings (9,12, 13,15), AP5 did not affect footshock sensitivity. There were no significant differences between groups in the flinch (unpaired *t*-test, t = -0.98, P = 0.34) or the jump (unpaired *t*-test, t = -0.09, P = 0.93) nociceptive thresholds.

Discussion

The results of the present report show that both pretraining and post-training, but not pretest, infusion of AP5 into the basolateral amygdala blocked retention of a step-down inhibitory avoidance task in rats. The effects of AP5 were not due to alterations in training session performance or footshock sensitivity. Furthermore, previous data from our laboratory have shown that infusion of AP5 into the BLA does not affect locomotor activity assessed by the number of crossings and rearings made during exploration of an open field (15).

The results suggest that intra-amygdala AP5 impaired the formation of memory for inhibitory avoidance. This is consistent with previous reports showing that AP5 infused into the amygdala impairs memory of several types of fear-motivated conditioning, namely, inhibitory avoidance (7-9,11,15), contextual fear conditioning (5,12), and conditioned fear-potentiated startle (4,13). These results are consistent with the view that an NMDA receptor-dependent neural plasticity mechanism such as LTP in the amygdala is involved in fear memory (2,10,12-14). However, extensive evidence suggests that the amygdala is not a critical site for acquisition

and storage of fear-motivated memory (1,3, 16-20). Thus, it is possible that AP5 induced nonspecific effects such as interfering with other neurotransmitter systems rather than blocking LTP induction (9), or that AP5 interfered with basal synaptic transmission in the BLA (2). Furthermore, the fact that AP5 infused into the amygdala impairs fear memory may be consistent with the view that NMDA receptor activation within the amygdala affects the regulatory activity of the amygdala on memory storage in other brain areas (1).

The finding that immediate post-training intra-amygdala infusion of AP5 impaired inhibitory avoidance retention suggests that amygdaloid NMDA receptors are involved in the early consolidation phase of memory for that task. This is consistent with previous reports showing the effects of post-training AP5 on inhibitory avoidance retention (7,8, 11). However, this is in contrast to the lack of effect of intra-amygdala infusions of AP5 on contextual fear conditioning when the drug is given immediately after training (12). This discrepancy might be due to differences in training procedures (for example, number of trials and training period) between the two tasks.

The lack of effect of the pretest infusion of AP5 suggests that amygdaloid NMDA receptors are not involved in the expression of memory for inhibitory avoidance. This is consistent with previous reports showing that intra-amygdala AP5 does not affect expression of fear-potentiated startle (4,6,13) or step-through inhibitory avoidance (11). However, it disagrees with the finding by Maren et al. (12) that infusion of AP5 into the BLA impairs expression of contextual fear conditioning. The possibility that AP5 affects basal synaptic transmission in the BLA has been raised as an interpretation for the finding by Maren et al. (12) that AP5 blocked the expression of fear conditioning (2,9).

In summary, the present report shows that both pretraining and post-training, but not pretest, infusion of AP5 into the basolateral amygdala prevents retention of inhibitory avoidance, suggesting that amygdaloid NMDA receptors are involved in formation, but not in the expression, of this type of fear-motivated memory. This does not imply that the role of NMDA receptors in the BLA is to mediate long-term storage of fear-motivated memory in the amygdala.

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