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Pretraining Infusion of DSP-4 into the Amygdala Impaired Retention in the Inhibitory Avoidance Task: Involvement of Norepinephrine but not Serotonin in Memory Facilitation

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Abstract

The present study investigated the involvement of amygdala noradrenergic (NE) and serotonergic (5-HT) systems in memory storage processing. Rats bearing chronic cannulae in the amygdala were trained on a one-trial inhibitory avoidance task and tested for retention 24 hrs later. Five days prior to training, rats received intra-amygdala infusion of vehicle or various doses of N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP-4)–a NE-specific neurotoxin when given peripherally. Results showed that pretraining intra-amygdala infusion of 10.0 μg or 30.0 μg of DSP-4 impaired retention. Further, 30.0 μg of DSP-4 also abolished the memory enhancing effect of epinephrine (E) injected peripherally. However, local infusion of DSP-4 depleted not only NE but also 5-HT and DA substantially. Subsequent experiments found that the retention deficit induced by 30.0 μg of DSP-4 could be ameliorated by 0.2 μg NE but not by 5-HT at a wide range of doses infused into the amygdala shortly after training, which ascribed the deficit to depletion of NE. After protecting the 5-HT terminals by a pretreatment of fluoxetine (15.0 mg/kg), pretraining intra-amygdala infusion of 30.0 μg DSP-4 shifted the memory-enhancing dose of E from 0.1 mg/kg to 1.0 mg/kg. In contrast, pretraining intra-amygdala infusion of 15.0 μg 5,7-dihdroxytryptamine (5,7-DHT) or DSP-4 with a pretreatment of desipramine (DMI, 25.0 mg/kg×2) to protect NE terminals failed to impair retention or attenuate the memory enhancing effect of 0.1 mg/kg E injected peripherally. These findings, taken together, suggest that the memory modulatory effect of peripheral E involved, at least partially, the amygdala NE system.

Key Words: epinephrine, aversive learning, memory, 5,7-DHT, rats

Introduction

The central noradrenergic system has been implicated in memory processing. Altering central noradrenergic functions influenced acquisition or retention of various learned responses (40). Intracerebroventricular (icv) injections of diethyldithiocarbamate (DDC), which inhibits the dopamine-β-hydroxylase (DBH), impaired retention of an inhibitory avoidance response (42) and this deficit was readily attenuated by posttraining icv injections of norepinephrine (NE) (48). Lesions of the locus coeruleus, from which NE fibers arise, potentiated an otherwise non-apparent amnesic effect of delayed electroconvulsive shock (52) or attenuated the memory enhancing effect of vasopressin (30).

Recent evidence further indicated that memory storage processing involves NE activity in specific forebrain regions. Depletion of NE in the prefrontal cortex of monkeys with 6-hydroxydopamine (6-OHDA) impaired their performance in a delayed alternating task and the deficit was ameliorated by an

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α_2 agonist – clonidine (4). In rats, this and other laboratories have shown that posttraining intra-amygdala infusion of noradrenergic agonists or antagonists enhanced or impaired retention, respectively, in various tasks (16, 31, 36). Training experience of certain types has been shown to affect NE metabolism in brain regions including the amygdala (21). Such results, taken together, suggest that NE may be normally released into the amygdala during training and play an important role in consolidating memory for newly acquired experience.

Extensive evidence also showed that retention in various tasks could be altered by posttraining systemic injection of epinephrine (E) (19), a hormone which may work as an endogenous memory modulator (40). However, peripheral E contributes little to the central E content under normal circumstance (44). Thus, the intriguing issue of how E affects memory formation in the brain instigated a variety of conjectures (8, 20). Recent findings from this laboratory suggest a possibility that the influence of peripheral E on memory may be mediated by the amygdala NE system. Posttraining intra-amygdala infusion of a β-noradrenergic blocker–propranolol–readily attenuated the memory enhancing effect of peripherally administered E in either normal or adrenal demedullated rats (31, 36). Further, suppressing the locus coeruleus activity by local infusion of clonidine impaired retention and abolished the memory enhancing effect of peripheral E (33). These findings were consistent with a possibility that E administered peripherally may somehow activated NE fibers innervating the amygdala and hence released NE which modulated memory formation processes.

According to the above suggestion, one may expect that depletion of NE in the amygdala would not only impair retention but also attenuate the memory enhancing effect of E. A neurotoxin N-2-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) injected peripherally could cause substantial and selective depletion of central NE, which lasted almost permanently (29). Evidence suggested that DSP-4 interacted initially with uptake sites on the NE terminals (11), preferentially those projecting from the locus coeruleus (29). However, there have been conflicting results for the effects of DSP-4 on learning and memory. Some investigators reported that DSP-4 treated rats showed an acquisition deficit in the two-way active avoidance and sensory preconditioning tasks (1, 2, 3, 6) and DSP-4 treated chicks had deficits in imprinting (9). Other investigators have failed to demonstrate an effect of DSP-4 on learning and memory in various tasks including the inhibitory avoidance task (5, 7, 46). In a previous experiment, we also found that pretraining intra-amygdala infusion of 3.0 μg DSP-4 failed to impair retention in the inhibitory avoidance task (36). However, in that study effects of higher doses were not tested and the control group had relatively low retention performance which might impede demonstration of further impairment due to floor effects. This study was thus designed to examine whether in the inhibitory avoidance task, intra-amygdala infusion of DSP-4 at higher doses would impair memory under training conditions in which the controls showed substantial retention. In addition, we also investigated whether such a treatment would attenuate the memory enhancing effect of peripheral E and if it did, whether the effect was due to NE depletion.

Materials and Methods

Subjects

Male Sprague-Dawley rats (60-70 days old, 250-300 grams) purchased from the local breeding center, were individually housed upon arrival and maintained on a 12/12 light-dark cycle (lights on at 7:00 am) with food and water ad libitum.

Surgery

Approximately three weeks after arrival, all rats were subjected to stereotaxic brain surgery. Under sodium pentobarbital (50 mg/kg) anesthesia, 23 Gauge stainless steel thin-wall cannulae (15 mm long) were implanted bilaterally into the amygdala in the animals. The cannula tip was aimed at the dorsal surface of the amygdala complex (A.P.-1.0 mm from the bregma, M.L. ±4.5 mm from the midline, D.V. -7.0 mm below the skull surface, the nose bar was at 5.0 mm above the interaural line). Two cortical screws serving as anchors were implanted over the right frontal and left posterior cortices. The cannulae were affixed on the skull with dental cement. A stylet (made from 00 insect pins) was inserted into each cannula to maintain patency.

Behavioral Tasks

Inhibitory Avoidance Task. Two weeks following the surgery, rats were trained on a one-trial step-through inhibitory avoidance task. The procedure has been adopted by previous studies (35). Briefly, the apparatus consisted of a trough-shape alley divided by a sliding door into an illuminated safe compartment and a dark shock compartment. The rat was placed into the safe side facing against the door. As the rat turned around, the door was opened. After the rat entered the dark compartment, the door was closed and a footshock was administered. The shock intensity, calculated as the root-mean-square of

sinusoidal currents, was set at 1.2 mA and 1.2 s. The rat was removed from the alley about 5 s after receiving the shock, administered the appropriate posttraining treatments, and returned to its home cage. On the retention test given 24 hrs later, the rat was placed into the illuminated compartment as on the training trial and the latency to step into the dark side was recorded as the measure of retention. Rats which did not enter the dark within 600 s were removed from the alley and assigned a ceiling score of 600.

Open Field Task. Some of the rats were tested for locomotor activity two weeks after the inhibitory avoidance task. The apparatus was a platform elevated 65 cm above the ground. The dimension of the field was 76 cm × 76 cm and divided into 16 (4 × 4) equal squares. The animal was placed on a corner of the field and an experimenter recorded the number of line crossings made in a period of 5 min.

Flinch-Jump Task. To assess the effect of intra-amygdala administered DSP-4 on shock sensitivity of animals, the flinch-jump thresholds in response to electric shocks were determined in some vehicle and DSP-4 injected rats two weeks after the inhibitory avoidance task. A series of 10 shocks, ranging from 0.1 to 1.0 mA at 0.1 mA intervals, was administered to the animal in an illuminated alley. Each rat received 6 shock series: 3 in a descending order and 3 in an ascending order. The inter-shock and the inter-series intervals were 1 min. A flinch response was defined as a sudden startle movement of the rat in receiving shocks, while a jump response was scored when the rat ran or lifted its hind legs from the electrified floor. The "flinch threshold" was defined as the shock intensity at which 50% of the rats' responses were no reaction and 50% of them were "flinch", while the "jump threshold" was defined as the shock intensity at which 50% of the rats' responses were "flinch" and 50% were "jump".

Drugs and Drug Administration

Desipramine (DMI), 5,7-dihydroxytryptamine (5,7-DHT) and norepinephrine hydrochloride were obtained from Sigma (St. Louis, Mo). DSP-4 was obtained from Hoechst. Fluoxetine (FLX) was obtained from Lilly. Epinephrine (E) was obtained from a local pharmaceutical company. Drugs for central administration were dissolved into a special brain buffer solution (13), which in 100 ml, contained 0.9 g NaCl, 4.05 ml of 0.2 M Na_2HPO_4 and 0.95 ml of 0.2 M $NaH_2PO_4 \cdot H_2O$. Drugs for peripheral injection were dissolved into saline or water. Animals received intra-amygdala infusion of vehicle (Veh), DSP-4 or 5,7-DHT at least 5 days before inhibitory avoidance training. In some experiments, rats were pretreated with FLX or DMI before receiving infusion of DSP-4 or 5,7-DHT. Behavioral assessment was performed from 5 to 7 days after infusion of toxin, at which time significant depletion of the neurotransmitter was present according to a previous report (5). The intra-amygdala infusion was administered through a 30 Gauge dental needle connected to a 10 µl Hamilton microsyringe by 0.5 meter polyethylene tubing (PE-20). The injection needle was bent at a length such that, when inserted into the cannula, the needle tip would protrude 2.0 mm beyond the tip of the cannula. Drug solution was introduced into the PE tubing and delivered into the amygdala manually at a rate of 1 µl/min. For DSP-4 or 5,7-DHT infusion, 2 µl was administered into each side, for NE infusion, 1 µl per side was given. E was injected subcutaneously (s.c.).

Histology

At the conclusion of the experiment, some rats were sacrificed with an overdose of sodium pentobarbital and were perfused through the heart with 0.9% saline followed by 10% formalin. The brain was removed from the skull, stored in formalin for at least 48 hrs and then sectioned into 40-µm slices. The brain slices through the cannula tract were stained with cresyl violet and the locations of cannula tips were examined on the atlas of Paxinos and Watson (43).

Monoamine Assays

To assess the degree of monoamine depletion in the amygdala and other brain regions after the DSP-4 or 5,7-DHT treatment, some animals were sacrificed by decapitation after behavioral tasks. Tissues from the amygdala region were dissected out, frozen with dry ice and stored at -75°C until assay. The monoamine assay followed a procedure described elsewhere (26). Briefly, the tissue was homogenized in HCl with 100 ng of 3,4-dihydroxybenzylamine (DHBA) serving as an internal standard and monoamines were extracted by a butanol method. Twenty microliters of the aqueous extract were injected through a Rheodyne injector into a 25 cm × 4.6 mm Ultrasphere C-18 reversed phase column. The mobile phase was a 0.04 M sodium citrate/citric acid buffer (pH 5.0) containing 0.6 % tetrahydrofuran with 1 ml/min flow rate. Monoamines were detected by an electrochemical detector (BAS, LC-4B, West Lafayette) with a glassy carbon electrode. The applied voltage was set at 600 mV versus Ag/AgCl reference electrode. Ratio of peak heights for monoamines to the internal standard was compared for samples and external standards taken through the entire extraction procedure.

Table 1. Effects of Intra-amygdala Infusion of DSP-4 on Monoamine Contents of the Amygdala. (mean ± 1 S.E. ng/g of wet tissue)

Treatment	NE	DA	5-HT
Veh (n = 13)	537.1±19.1	513.8 ± 45.4	1389.2 ± 57.1
3.0 µg DSP-4 (n = 13)	452.9±15.6*	440.9 ± 161.6	1031.8 ± 65.9*
30.0 µg DSP-4 (n = 18)	335.6±11.6**	325.7 ± 26.7**	729.2 ± 28.3**

*p < 0.01; **p < 0.001 two-tailed t-tests

Statistics

In the inhibitory avoidance experiment, the distribution of retention scores was truncated at 600, thus medians and interquartile ranges were used to denote the central and dispersion tendencies, respectively. Nonparametric statistics were used to analyze the data. The Kruskal-Wallis one-way analysis of variance was used to detect overall differences among the groups, followed by paired comparisons with Mann-Whitney two-tailed U-tests. For the data of open field activity, shock sensitivity or monoamine levels, means and standard errors were used to denote the central and dispersion tendencies, respectively; and differences between groups were detected by independent t-tests.

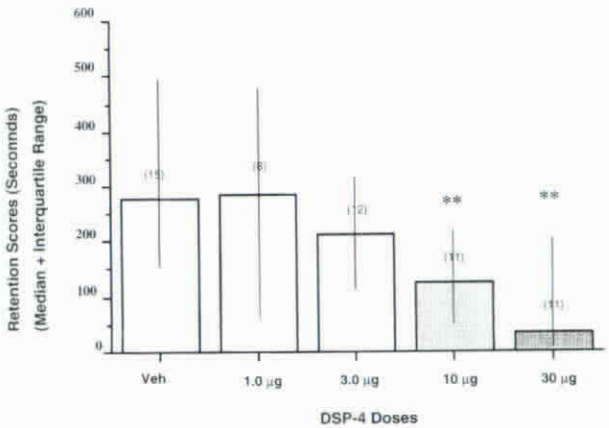

Fig. 1. Effects of intra-amygdala infusion of DSP-4 on retention in an inhibitory avoidance response. **p<0.01 different from the Veh group.

Results

Experiment I. Effect of Pretraining Intra-Amygdala Infusion of DSP-4 on Retention

To examine whether depletion of NE in the amygdala would impair retention of the inhibitory avoidance response, five groups of rats received bilateral intra-amygdala infusion of Veh or DSP-4 at the doses of 1.0, 3.0, 10.0 or 30.0 µg. Five days following the infusion, animals were trained on the task and then tested as previously described. Some of the rats were subjected to the open field test or the shock sensitivity test two weeks later. Retention in various groups is shown in Figure 1. A one way analysis of variance revealed a significant difference among groups ($H'(4) = 12.42$, $p < 0.02$). Multiple comparisons indicated that rats receiving either 10.0 µg or 30.0 µg of DSP-4 had significantly lower retention scores than the Veh group ($U = 42$ & 38.5, respectively; $p < 0.02$). While rats receiving 3.0 µg of DSP-4 tended to have lower retention scores than rats receiving Veh, the difference failed to reach statistical significance.

The number of line crossings in the open field test was 47.2 ± 3.0 and 43.7 ± 4.0 (mean ± 1 s.e.) for the Veh and DSP-4 animals, respectively. The difference was not statistically significant. The "flinch" threshold for the Veh and DSP-4 animals was 0.19 ± 0.02 mA and 0.23 ± 0.06 mA, respectively. The "jump" thre-shold for the Veh and DSP-4 animals was 0.33 ± 0.04 mA and 0.32 ± 0.03 mA, respectively. No significant difference was found in either threshold.

Some animals were sacrificed after all behavioral tests for monoamine assays to evaluate the degree of depletion. The extent of monoamine depletion resulted from 3.0 µg and 30.0 µg DSP-4 is shown in Table 1. As indicated in the table, intra-amygdala infusion of 30.0 µg DSP-4 induced depletion of not only NE but also DA and 5-HT.

Experiment II. Effect of Pretraining Intra-amygdala Infusion of DSP-4 on the Memory Enhancing Effect of E

This experiment assessed whether pretraining intra-amygdala infusion of a high dose of DSP-4 would attenuate the memory enhancing effect of peripheral E. Eight groups of rats were pretreated with intra-amygdala infusion of Veh or 30.0 µg DSP-4 five days before training. Immediately after training, they received an injection of saline or E at the dose of 0.001, 0.01 or 0.1 mg/kg.

Retention was tested 1 day later. Retention scores for various groups are shown in Figure 2. It is

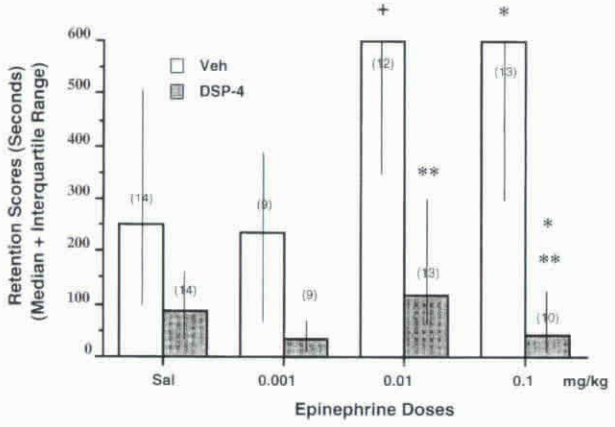

Fig. 2. Effects of posttraining s.c. injection of E on retention in the inhibitory avoidance task for rats pretreated with intra-amygdala infusion of 30.0 µg DSP-4. +0.05 < p < 0.075, *p < 0.05, both different from the Veh/Sal group; **p < 0.01, ***p < 0.001, both different from the correspondent Veh groups.

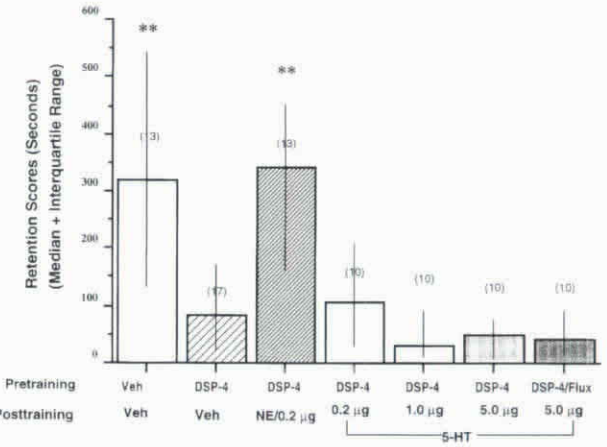

Fig. 3. Effects of intra-amygdala infusion of NE or 5-HT on retention in the inhibitory avoidance task for rats pretreated with intra-amygdala infusion of 30.0 µg DSP-4. **p < 0.01 different from the groups receiving posttraining infusion of Veh or 5-HT.

apparent from the figure that posttraining systemic injection of E caused dose-dependent retention enhancement and that was abolished in the DSP-4 pretreated rats. The data for the Veh-pretreated groups and the DSP-4 pretreated groups were analyzed separately by two analyses of variance. There were significant differences among the various Veh-pretreated groups ($H'(3) = 11.1$, $p < 0.05$). Further comparisons indicated that the group receiving 0.1 mg/kg E had significantly better retention scores than the saline controls ($U = 44$, $p < 0.05$). The group receiving 0.01 mg/kg E also showed good retention scores, however, the difference between it and the control group only approached statistical significance ($U = 47.5$, $0.05 < p < 0.075$). The group receiving 0.001 mg/kg E did not differ from the control.

In contrast, no overall significant difference was detected among the various DSP-4 pretreated groups ($H'(3) = 6.8$, $p < 0.08$). Rats receiving various doses of epinephrine after training did not differ from the saline controls in retention scores. For rats given posttraining injections of saline, the DSP-4 pretreated group had lower retention scores than the Veh pretreated group, and the difference approached statistical significance ($U = 58$, $0.05 < p < 0.07$). Further, groups pretreated with DSP-4 and later receiving 0.01 or 0.1 mg/kg E had significantly poorer retention than the correspondent groups pretreated with Veh ($U = 23$ & 8, respectively; $p < 0.01$).

Some rats were sacrificed after the behavioral test for monoamine assays to evaluate the degree of depletion. In replicating findings of the previous experiment, intra-amygdala infusion of 30.0 µg DSP-4 induced depletion of monoamines, the levels for the control and DSP-4 groups were 592.2 ± 42.4 ng/g and 289.4 ± 42 ng/g for NE, 559 ± 50.7 ng/g and 421.2 ± 52.6 ng/g for DA, as well as 1152.1 ± 85.7 ng/g and 616.5 ± 52.1 ng/g for 5-HT.

Experiment III. Posttraining Intra-Amygdala Infusion of NE Attenuated the DSP-4 Induced Amnesia

In view of the above results, it is necessary to demonstrate that posttraining intra-amygdala infusion of NE were able to attenuate the DSP-4 induced retention deficit, otherwise the observed impairment could be due to depletion of 5-HT or DA. Rats received pretraining intra-amygdala infusion of Veh or 30 µg DSP-4. These rats were trained as described previously. Immediately after training, the Veh pretreated rats and a group of DSP-4 rats received intra-amygdala infusion of Veh, while other rats received intra-amygdala infusion of 0.2 µg NE or 0.2, 1.0 or 5.0 µg 5-HT. To evaluate whether 5-HT might be equipotential to NE in attenuating retention deficits induced by NE depletion, an extra group received an i.p. injection of 15.0 mg/kg fluoxetine (FLX) 30 min before infusion of DSP-4. This group received posttraining intra-amygdala infusion of 5.0 µg of 5-HT. Retention performance of groups receiving different pretaining/posttraining treatments is shown in Figure 3. The effects of NE and 5-HT on attenuating DSP-4 induced deficit were analyzed by two separate one-way analyses of variance. One analysis revealed significant differences among the Veh/Veh, DSP-4/Veh and DSP-4/NE groups ($H'(2) = 11.1$, $p < 0.005$). Consistent with the previous results, intra-amygdala infusion of DSP-4 impaired retention, the DSP-4/Veh group had significantly lower retention than the Veh/Veh group ($U = 47$, $p < 0.01$). Posttraining intra-amygdala infusion of 0.2 µg NE readily ameliorated this retention deficit, the DSP-4/

NE group had retention scores significantly better than the DSP-4/Veh group ($U = 40$, $p < 0.005$) but not different from the Veh/Veh control group.

A second analysis of variance revealed significant differences among the controls and the DSP-4 groups treated with 5-HT ($H'(5) = 18.23$, $p < 0.005$). Posttraining intra-amygdala infusion of various doses of 5-HT failed to improve retention performance in the DSP-4 infused rats: The DSP-4 pretreated group infused with 0.2, 1.0 or 5.0 μg 5-HT had retention scores lower than the Veh/Veh group ($U = 30$, 11 & 19; $p < 0.05$, 0.001 & 0.005, respectively), but not significantly different from the DSP-4/Veh group. For the FLX+DSP-4 group, in which presumably NE was depleted more than 5-HT, 5.0 μg 5-HT failed to attenuate the retention deficit, the FLX+DSP-4/5-HT group still had significantly lower retention scores than the Veh/Veh control group ($U = 13$, $p < 0.05$).

Experiment IV. E Failed to Enhance Retention in Rats Pretreated with DSP-4 plus Fluoxetine

The present experiment examined whether attenuation of the E-induced retention enhancement was due to depletion of NE or other monoamines after local infusion of DSP-4 as shown by the above results. In addition, effects of E at a wider dose range were explored to detect possible dose-response curve shifts after the DSP-4 treatment. A batch of rats received intra-amygdala infusion of Veh or DSP-4 as previously described. However, 1 hr before the infusion, all rats were pretreated with an i.p. injection of 15.0 mg/kg FLX, which was used to protect 5-HT terminals from the neurotoxicity of local DSP-4 infusion. All rats received the inhibitory avoidance training 5 days after DSP-4 infusion. Immediately after training, rats received an injection of either saline or 0.01, 0.1, 0.5 or 1.0 mg/kg E. Retention performance was tested 24 hrs later.

Results are shown in Figure 4. As found in the previous experiment, E still enhanced retention in the FLX+Veh pretreated groups. This effect was attenuated by the treatment of DS-4 plus FLX: E enhanced retention only at a higher dose in the FLX+DSP-4 rats. The data were analyzed separately by two analyses of variance. An overall significant difference was detected among the FLX+Veh pretreated groups ($H'(4) = 13.5$, $p < 0.01$). Further paired comparisons indicated that in comparison with the saline group, 0.1 mg/ kg E enhanced retention ($U = 5$, $p < 0.001$). While 0.01 mg/kg E appeared to have an enhancing effect, the difference was not significant. Doses higher than 0.1 mg/kg had no significant effect. The DSP-4 plus fluoxetine pretreatment tended to impair retention in the saline injected rats: The

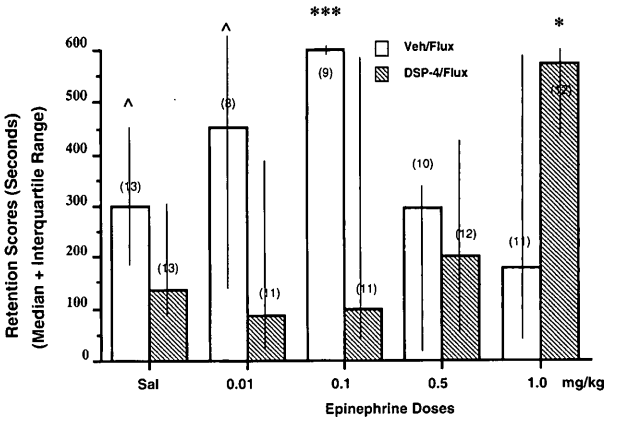

Fig. 4. Effects of posttraining s.c. injections of E on retention in the inhibitory avoidance task for rats receiving intra-amygdala infusion of Veh- or DSP-4 plus pretreatment of fluoxetine (FLX). ^ different from the correspondent FLX+DSP-4 group $0.05 < p < 0.075$. * $p < 0.05$ different from the correspondent Sal controls. ***$p < 0.001$ different from the Sal controls and the correspondent FLX+DSP-4 group receiving the same dose of E.

FLX+DSP-4/Sal group tended to have lower retention than the FLX+Veh/Sal group ($U = 49$, $0.05 < p < 0.07$). In the DSP-4 plus FLX pretreated rats, while an overall significant difference was not detected ($H'(4) = 7.3$, $p > 0.10$), paired comparisons indicated that the FLX+DSP-4 group receiving 1.0 mg/kg E had significant better retention scores than the correspondent saline group ($U = 37$, $p < 0.05$). Comparisons between the FLX+Veh groups and the FLX+DSP-4 groups receiving the same dose of E revealed that for rats receiving 0.1 mg/kg E, the FLX + Veh group had significant better retention than the FLX+DSP-4 group ($U = 15$, $p < 0.005$). A similar trend was also present in rats receiving 0.01 mg/kg E, but the difference only approached statistical significance ($U = 22$, $0.05 < p < 0.07$). The difference between two groups receiving 1.0 mg/kg E failed to reach statistical significance ($U = 40$, $p > 0.10$).

The amygdala of some rats in Experiment III and IV were assayed for depletion of monoamines. Fluoxetine did not provide full protection for serotonergic fibers in the amygdala, however, it greatly reduced the extent of 5-HT depletion induced by local infusion of DSP-4. Intra-amygdala infusion of DSP-4 plus pretreatment of FLX depleted 46% of NE (472 ng/g vs. 256 ng/g), 27% of DA (467 ng/g vs. 353 ng/g), and 18% of 5-HT (976 ng/g vs. 800 ng/g) in the amygdala.

Experiment V. Pretraining Intra-amygdala Infusion of 5,7-DHT Failed to Attenuate the Effect of Peripheral E

This experiment investigated whether specific depletion of 5-HT in the amygdala impaired retention

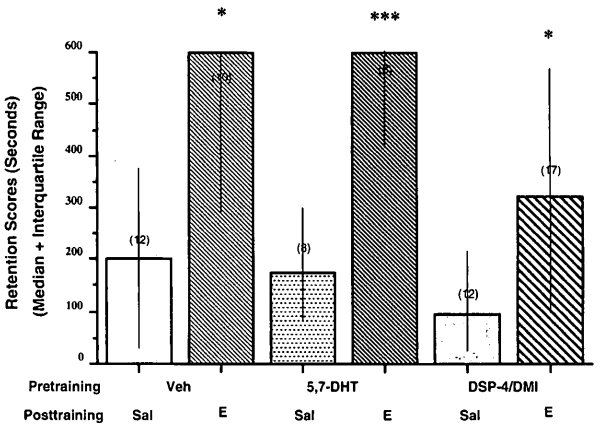

Fig.5. Effects of posttraining s.c. injection of E on retention in the inhibitory avoidance task for DMI-pretreated rats receiving intra-amygdala infusion of Veh, 5,7-DHT or DSP-4. *$p < 0.05$, different from the Veh/Sal group, ***$p < 0.001$, different from the correspondent Sal groups.

and abolished the memory enhancing effect of E. Six groups of rats received intra-amygdala infusion of Veh, 15.0 μg 5,7-DHT or 30.0 μg DSP-4 per side. To protect NE terminals, two doses of DMI (25 mg/kg) were injected i.p. 4 hrs and 1 hr prior to the central administration of neurotoxin. Rats were trained on the task with a 0.9 mA/1 s footshock five days after neurotoxin infusion. Immediately after training, half of the rats received an enhancing dose of E (0.1 mg/kg, s.c.), while the other half received saline. Retention was tested 24 hrs later.

Results for various groups are shown in Fig. 5. Consistent with previous findings, immediate posttraining injections of 0.1 mg/kg of E enhanced retention performance in the Veh pretreated rats (DMI+Veh/E group vs. DMI+Veh/Sal group, $U = 24$, $p < 0.05$). Infusing 5,7-DHT into the amygdala failed to attenuate the memory enhancing effect of E. Immediately posttraining injections of E still significantly improved retention in the 5,7-DHT treated rats (5,7-DHT/E vs. 5,7-DHT/Sal, $U = 4$, $p < 0.002$). In the DMI pretreated rats, intra-amygdala infusion of DSP-4 did not completely abolished the retention enhancing effect of peripheral E, the DMI+DSP-4/E group showed better retention than the DMI+DSP-4/Sal group ($U = 55$, $p < 0.05$). However, in the DMI+DSP-4 group, E failed to induced as much enhancement of retention as in the other two groups. Retention scores of the DMI+DSP-4/E group were significantly lower than those of the collapsed DMI+Veh/E and DMI+5,7-DHT/E group ($U = 87$, $z = 2.3$, $p < 0.05$).

The DMI+5,7-DHT treatment caused about 74% depletion of 5-HT (1489 ng/g tissue vs. 397 ng/g tissue) and about 25% depletion of DA (359 ng/g vs. 268 ng/g), but had little effect on the NE concentration of the amygdala. DMI did not provide complete protection of the NE terminals from DSP-4. In the amygdala of rats with DMI pretreatment, DSP-4 still caused about 30% depletion of NE (476 ng/g vs. 332 ng/g) and substantial depletion of DA (478 ng/g vs. 223 ng/g) as well as 5-HT (1593 ng/g vs. 967 ng/g).

Histology Verification

Figure 6 presents the locations of the cannula tips for a sample of animals in various coronal plains of a rat brain according to the atlas of Paxinos and Watson (43). The locations of the amygdala cannula tips appeared to spread over various nuclei of the amygdala. No correlation was found between the retention performance of an animal and the placement of the cannula within the amygdala.

Discussion

The present results could be summarized into two major findings: First, intra-amygdala infusion of DSP-4 before training induced a dose-dependent retention deficit in an inhibitory avoidance response, which was readily attenuated by posttraining intra-amygdala infusion of NE but not 5-HT. Second, pretraining intra-amygdala infusion of DSP-4 attenuated the memory enhancing effect of E administered into the periphery immediately after training. This attenuation effect was due to depletion of NE, but not 5HT, in the amygdala.

In the present study, DSP-4 was administered into the amygdala prior to the training experience. Therefore, the observed memory impairing effect could have been caused by influences of the drug on perceptual, motor or motivational processes during the phase of acquisition or recollection. This interpretation seems unlikely in view of the present results that pretraining intra-amygdala infusion of DSP-4 failed to affect locomotor activities in the open field test or shock sensitivity in the flinch-jump test. Similar findings have been reported previously (3, 46). In addition, the complete attenuation of the DSP-4 induced deficit by posttraining infusion of NE was also incompatible with the interpretation that the deficit could be due to altered perceptual, motor or motivational functions in the progress of training or testing. Such results ruled out as well a possibility that the observed memory deficit was caused by neuronal death in the amygdala after the DSP-4 treatment.

To the best of our knowledge, very few studies applied DSP-4 directly into the brain. In contrast to the peripheral administration of DSP-4 that caused

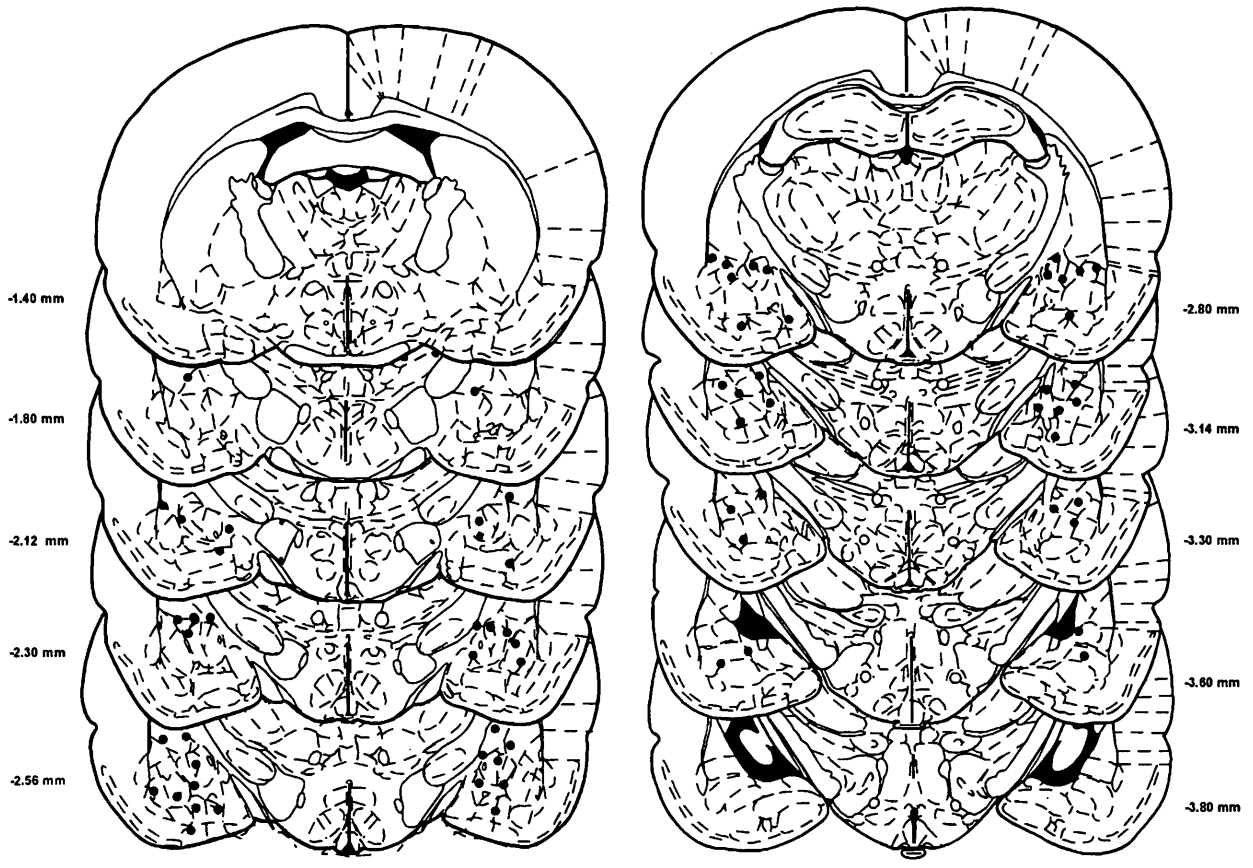

Fig. 6. Distribution of cannula tips in the amygdala from a sample of rats depicted in a series of coronal plains from bregma -1.80 mm to -3.80 mm based on the atlas by Paxinos and Watson (43).

rather selective depletion of NE in certain brain regions, this study showed that direct infusion of DSP-4 into the brain depleted not only NE but also DA and 5-HT to a certain degree, although it did not affect monoamine levels in non-target regions (data not shown). Apparently, local DSP-4 infusion gained better anatomical specificity at the expense of pharmacological specificity. The reason for the reduction of pharmacological specificity with centrally administered DSP-4 remains obscure and should be pursued in the future.

In addition to NE, 5-HT was also implicated in aversive learning (37, 45, 50). Thus, the observed memory deficit could be due to depletion of 5-HT or in combination with other neurotransmitter rather than depletion of NE alone. However, the results of Experiment III showed that posttraining intra-amygdala infusion of NE corrected the memory deficit readily. In contrast, posttraining intra-amygdala infusion of 5-HT at a wide range of doses failed to normalize retention in the DSP-4 treated animals. 5-HT could not act as a general memory enhancer because it did not attenuate the deficit caused by DSP-4 plus fluoxetine, which presumably caused more selective depletion of NE. As a matter of fact, our recent observation suggests that memory storage was inhibited by activating, instead of suppressing, the amygdala 5-HT function (34). A previous study has shown that long-term enhancement in the hippocampus, a form of neural plasticity which may underlie learning and memory, involved NE but not 5-HT (47).

The amygdala DA system has been implicated more in appetitive learning involving stimulus-reward association (22, 24) than in aversive learning. In contrast to the previous findings that systemic injections of DSP-4 had little effect on the central DA system (38), the present study showed that intra-amygdala infusion of DSP-4 also depleted DA to some extent. Nevertheless, the poor retention in the DSP-4 treated rats was completely rectified by posttraining intra-amygdala infusion of NE. Such findings corroborated the importance of NE in the DSP-4 induced effect, although they may not completely rule out a role of DA. The contribution of amygdala DA depletion to the inhibitory avoidance deficit induced by DSP-4 should be carefully examined in the future.

The present results are consistent with the notion

that depletion of NE in the amygdala may contribute significantly to the DSP-4 induced retention deficit. They provide further support, in addition to the existing ones (14, 16, 32), for the hypothesis that NE activity in the amygdala may be normally involved in memory consolidating processes (41). An in vivo microdialysis study has shown that NE was indeed released in the amygdala by footshock training experience (17). Electrophysiological studies have shown that in the amygdala slice preparation, stimulating β-receptors increased excitatory neurotransmission (25) and induced long term enhancement of EPSP (23). The plastic changes in the amygdala induced by NE or other factors could be critical for induction of the neural change elsewhere in the brain directly underlying formation of memory trace, as proposed by Weinberger (51) for retuning receptive band of some cortical auditory neurons during fear conditioning.

In contrast to the present findings, several previous studies reported that peripheral injections of DSP-4 (50 mg/kg or 100 mg/kg) did not affect retention performance in the inhibitory avoidance task (3, 5). It should be noted that while peripherally administered DSP-4 readily invaded NE terminals in the central nervous system (29), the resulted NE depletion was by no means uniform in various regions of the brain. The toxin administered as such caused approximately 90% of NE depletion in the hippocampus and the frontal cortex but spared a substantial proportion of the hypothalamic and amygdala NE fibers (5, 29). Therefore, the lack of a significant amnesic effect of DSP-4 as shown by previous studies may be related to insufficient depletion of NE in the amygdala. Consistent with this suggestion is the present finding that 3.0 μg of DSP-4, which depleted approximately 23% of NE in the amygdala, failed to affect retention performance significantly.

The present study found that intra-amygdala infusion of DSP-4 attenuated memory-enhancing effect of peripheral E. Because DSP-4 by itself had amnesic effects as shown by Experiment I, the attenuation may be due to summation of two effects opposite in direction and thus bear no relevance to the underlying mechanism (49). This is an implausible interpretation, because our previous study found that DSP-4 at a dose by itself not affecting memory attenuated both memory enhancing as well as memory impairing effects caused by different doses of E (36).

A major purpose of the present study was to investigate whether amygdala NE terminals mediated the influence of peripheral E on memory. Because intra-amygdala infusion of DSP-4 also depleted DA and 5-HT in the amygdala, the effect of E on memory could rely on transmitters other than NE. However, the attenuation persisted even if the 5-HT terminals had been protected, at least partially, by pretreatment of the serotonergic reuptake blocker fluoxetine. Moreover, depletion of 5-HT by local infusion of 5,7-DHT, which had very little effect on the amygdala NE concentrations failed to attenuate the memory facilitation produced by peripheral E injections. While a role of DA in mediating the E effect was not directly tested, yet across various experiments in which DA was depleted to various extents, no correlation was detected between the degree of DA depletion and the magnitude of the attenuation effect. For example, in Experiment III or IV, rats receiving DSP-4 or DSP-4 plus FLX pretreatment showed less than 30% depletion of DA on which the E effect was attenuated. Yet in Experiment V, rats receiving DSP-4 plus DMI pretreatment showed more than 50% depletion of DA on which the E effect was not much attenuated. Thus the integrity of amygdala DA system appeared not to bear a tight relationship with the influence of peripheral E on memory, although this issue remains to be clarified experimentally in the future.

The present study demonstrated that after depleting NE levels in the amygdala, the memory enhancing effect of an otherwise effective dose was attenuated. However, if a wider range of dose was tested, it was found that the memory enhancing effect of E shifted to a higher dose that was not enhancing in otherwise non-treated animals (19). Several reasons may be proposed to account for the shift of memory enhancing effects to a higher dose of E. Even at a dose of 30.0 μg, DSP-4 depleted no more than 50% of NE in the amygdala. Thus, the survived amygdala NE terminals may still be activated if a higher dose of E was given in the periphery. Further, the memory enhancing effect of E may involve NE fibers projecting not only to the amygdala but also to other forebrain regions (33). A higher dose of E may cause greater activation of NE activity in those regions, such as medial prefrontal cortex, which may compensate for the compromised amygdala NE function in induction of an enhancing effect. Finally, E may influence memory processing through multiple mechanisms. As DSP-4 blocked one of the mechanisms, others may be recruited to mediate the action of E at a higher dose. A possible one might be that through glycogenolysis, E could increase plasma levels of glucose that was shown to have memory facilitating effect (20).

Previous studies from this laboratory have shown that intra-amygdala infusion of NE or β-agonists not only enhanced retention but also ameliorated the memory deficit induced by removal of the adrenal medulla (31, 36). Intra-amygdala infusion of a β adrenergic blocker – propranolol–impaired memory and blocked the enhancing effect of E injected to normal or adrenal demedullated rats (31, 36). In view

of the evidence that DSP-4 destroyed NE terminals but spares the postsynaptic receptors (12), the present results excluded a possibility that E in the periphery may have somehow penetrated into the amygdala and acted directly on adrenergic receptors to affect memory processes (27). If this were the case, there should have been a left shift rather than a right shift in the dose response curve, because after destruction of NE terminals amygdaloid adrenergic receptors may be more sensitive.

The present findings, in conjunction with previous ones, support the hypothesis that the amygdala NE function, at least in part, mediates the influence of peripheral E on memory processes. Consistently, previous evidence has shown that peripheral injections of E altered forebrain NE levels (18) and NE release in the amygdala was highly correlated with plasma levels of E and NE (10). It remains to be elucidated how peripheral E could affect NE activities in the amygdala. The brainstem NE neurons such as those in the locus coeruleus have been shown to receive visceral inputs from the periphery (49). These nuclei provide NE innervation to the amygdala (15, 28). It is likely that peripheral E may excite the centripetal visceral afferents that in turn activate the NE nuclei in the pons or medulla and result in release of NE in the amygdala. Consistent with this conjecture is our findings that clonidine induced suppression of the locus coeruleus not only impaired retention but also attenuated the memory enhancing effect of peripheral E (33).

In conclusion, findings from the present study suggest that the amygdala NE system may mediate the memory modulatory effect of peripheral E, which is released in response to events that have special significance to the organism. That E could improve memory for those episodes preceding its release would guarantee better preservation of vital experiences in memory and may represent a natural device of how our daily experience is selected for long-term storage in the nervous system.

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