Posttraining Infusion of Norepinephrine and Corticotropin Releasing Factor into the Bed Nucleus of the Stria Terminalis Enhanced Retention in an Inhibitory Avoidance Task

K. C. Liang, Hsiu-Chen Chen and Der-Yow Chen

Department of Psychology
National Taiwan University
Taipei 106, Taiwan, ROC

Abstract

The present study investigated whether the bed nucleus of the stria terminalis (BNST) is involved in formation and retrieval of affective memory. Male Wistar rats with cannulae bilaterally implanted into the BNST were trained on a one-trial step-through inhibitory avoidance task. Shortly after training they received bilateral intra-BNST infusion of lidocaine, various noradrenergic drugs, or corticotropin releasing factor (CRF). Results showed that posttraining intra-BNST infusion of lidocaine impaired retention. Posttraining intra-BNST infusion of norepinephrine or the α_1 antagonist prazosin induced a dose- and time-dependent retention enhancement or deficit, respectively. The enhancing effect of norepinephrine was mimicked by the α_1 agonist phenylephrine, and antagonized by prazosin at a non-impairing dose. Posttraining intra-BNST infusion of the α_2 antagonist idazoxan or the β antagonist propranolol failed to affect retention. Posttraining intra-BNST infusion of CRF also enhanced retention in a dose-dependent manner. Various drugs infused shortly before testing did not significantly influence locomotor activity and retention. These findings, taken together, suggest that the BNST is involved in memory formation processes for affective experience and norepinephrine released in the BNST acting via α_1 receptors plays a critical role in this function.

Key Words: memory, fear conditioning, BNST, lidocaine, prazosin, phenylephrine, rats

Introduction

Extensive evidence implicates the amygdala in memory processing of affective information: Manipulation of amygdaloid functions shortly after training caused a time-dependent effect on retention in learning tasks that arouse strong emotion. Existing evidence suggests reliance of memory function of the amygdala on its various afferent-efferent pathways including the stria terminalis (ST). An early study has shown that pretraining lesions of the fornix/ST attenuated the retention enhancing effect of vasopressin injected peripherally (59). Subsequent studies showed that in an inhibitory avoidance task pretraining lesions of the ST caused negligible or mild retention deficits by themselves (30) but attenuated the memory impairing effect of posttraining subseizure electrical stimulation of the amygdala (29). These findings suggest a critical role of the ST in mediating memory modulatory influences of the amygdala to elsewhere in the brain.

The ST is also involved in the memory modulatory effect of other treatments: Posttraining injections of epinephrine into the periphery facilitated memory in the inhibitory avoidance task (31), and this enhancing effect was mediated by release of norepinephrine (NE) in the amygdala (33). Pretraining lesions of the ST attenuated not only the memory enhancing effect of peripherally injected epinephrine (28, 56) but also that of NE infused into the amygdala

Corresponding author: Dr. Keng Chen Liang, Department of Psychology, National Taiwan University, 1 Roosevelt Road, Sec 4, Taipei 106, Taiwan, ROC. E-mail: kcliang@ms.cc.ntu.edu.tw
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(34). Consistently, ST lesions blocked the memory enhancing effect of systemic injection of clenbuterol, which exerted its action through amygdaloid β receptors (22). In addition, ST lesions abolished the effects on memory of naloxone or β-endorphin (41), cholinergic drugs (21), CCK-8 (17), glucocorticoid (53) given systemically, as well as intra-caudate infusion of oxotremorine (47). Thus, integrity of the ST appears to be essential for various treatments to exert their influences on memory processes, either through the amygdala or elsewhere in the brain.

Amygdaloid efferents in the ST innervate various target regions including the bed nucleus of the ST (BNST) (13). This basal forebrain structure has been viewed as part of the extended amygdala (1) due to the similarity of its input-output patterns and physiological functions to those of the amygdala (23). All above findings considered together predicted a role of the BNST in memory processing of affective experience. However, this prediction by far has met with contradictory evidence. On the basis of the evidence that ST fibers contains met-enkephalin (57) which modulated BNST neuronal activity (10), a previous study showed that posttraining intra-BNST infusion of an opioid agonist levorphanol caused a memory deficit, and intra-BNST infusion of naloxone attenuated this deficit as well as that caused by subseizure electrical stimulation of the amygdala (35). These results suggested a role for the endogenous opioid of the BNST in modulating of memory formation.

On the other hand, Davis and his colleagues showed that lesions of the BNST abolished potentiation of acoustic startle caused by corticotropin releasing factor (CRF) or intense light flashes but had no effect on that caused by an otherwise neutral stimulus associated with electric shocks (26, 60). These data were consistent with those showing that expression of conditioned freezing and heart rate responses did not rely on the ST and its projection areas (24). Such results could be taken as evidence suggesting lack of a role of the BNST in formation and/or expression of conditioned fear responses.

These inconsistent results suggest that further study on the role of the BNST in learning and memory is warranted. The BNST receives dense innervation of noradrenergic fibers from the brain stem A1, A2 regions and locus coeruleus (37, 49). It contains the highest immunoreactivity of dopamine-β-hydroxylase in the brain (5) as well as abundance of α_{1a}, α_{1b}, α_2 and β receptors (12, 38, 58). Immobilization stress caused in vivo release of NE in the nuclei (46). The BNST also contains high densities of CRF immunoreactive cell bodies and neural processes, some of which are innervated by noradrenergic fibers (49). CRF is implicated in modulating emotional behavior (55): Avoidance of water stress activated expression of c-fos in the BNST, which was mediated through CRF (6). In view of the previous findings that infusion of NE or CRF into the amygdala or hippocampus enhanced memory (25, 27, 31, 39), it is of interests to investigate the role of CRF or NE, as well as its various subtypes of receptor, within the BNST in affective memory. To address this issue, experiments were designed in the present study to examine the effect of posttraining or pretest infusion of various adrenergic drugs or CRF into the BNST on retention of an inhibitory avoidance response, an aversive learning task widely adopted for assessing memory processing of emotional events (42).

Methods and Materials

Subjects

Male Wistar rats of 3 to 4 months old, weighing from 300 to 350 grams were used in this study. After being received from the National Breeding Center of Experimental Animals (Nankang, Taipei), they were individually housed in our animal facilities. 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. All experiments were carried out in accordance with Animal Research Guidelines described in Ethical Codes of Chinese Psychological Association.

Surgery

One month after arriving, rats were implanted with guide cannulae bilaterally into the BNST. They were anesthetized with injection of sodium pentobarbital (ip, 45 mg/kg). To prevent respiratory congestion, atropine sulfate (0.4 mg/kg) was given 10 min before the anesthetic. To implant cannulae into the BNST, the anesthetized rat was mounted on a DKI-900 stereotaxic instrument; the coordinates were AP. -0.9 mm, ML. ±1.6 mm and DV. -5.6 mm with the incisor bar set at -3.3 mm. Cannulae were made of 23 G stainless steel tubing with 0.33 mm inner diameter and 0.63 mm outer diameter at a length of 15 mm. Two jewelry screws were implanted over the right frontal and left posterior cortices serving as anchors. The whole assembly was affixed on the skull with dental cement. Rats were kept warm until resurrection from the surgery. They recuperated for at least two weeks before commencement of any behavioral experiments.

Behavioral Tasks

Inhibitory Avoidance. Rats were trained and

tested on a one-trial step-through inhibitory avoidance task with a procedure that has been adopted by many studies (36). Briefly, the apparatus was a trough-shape alley divided by a sliding door into a well-lit safe compartment and a dark shock compartment. The rat was placed into the lit side facing away from the door. As the rat turned around, the door was opened. After the rat stepped into the dark compartment, it received an inescapable footshock via a constant current shocker controlled by a timer (Lafayette Instruments, Model 80240 and Model 58010, Indiana, USA). The shock intensity was calculated as the root mean square of the sinusoidal alternating currents. Various experiments used different levels of shock intensity, which were chosen as optimal for detecting the effect of a given treatment based on previous findings and would be specified in each experiment. After administration of the shock, the rat was removed from the alley and returned to its home cage. In a retention test given 24 hrs later, the rat was reintroduced into the alley and the latency of stepping into the shock compartment with all four feet was taken as the retention score. If a rat did not step through in 10 min, the test trial was terminated and a ceiling score of 600 s was assigned.

Locomotor Activity. To assess the possible effect of some drugs on locomotion, a subset of rats were tested for locomotor activity after the inhibitory avoidance test. The rat was placed into an arena (30×30×50 cm) after intra-BNST infusion of drugs and its activity was videotaped for 15 min. These tape-recorded data were then analyzed by the Ethovision system (Noldus Information Technology, Netherland) for detection of horizontal movement. Cumulative distance traveled was calculated for each of the five 3-minute blocks.

Shock Sensitivity. In order to evaluate influences of the drugs on sensitivity to electric shocks, a subset of rats already tested in the inhibitory avoidance task were subjected to a shock startle test, in which the effects of altered sensory receptivity and motor reactivity could be dissociated (9). Briefly, rats after being infused with saline or various drugs were put into a startle apparatus (San Diego Instrument, San Diego, U.S.A.) with a continuous 55 dB background noise. After an acclimation period of 5 min, 45 startle trials were presented with an inter-trial interval of 30 s. Two types of stimuli were used to elicit startle: A type of stimuli dispensed from a programmable shocker (TI 30, Coulburn Instrument, San Diego, U. S.A.) contained 9 different intensities of electric shocks (0.1 s duration) ranging from 0 to 1.6 mA at incremental steps of 0.2 mA. The other type was white noise bursts at intensities of 95, 105 and 115 dB (40 ms duration). Each session contained three blocks of trials, and each block was composed of 6 acoustic trials (2 trials at each sound level) followed by 9 shock trials (1 trial at each shock intensity). Different intensities of each stimulus modality were presented in a quasi-random order within the separated phases in a series. The total time elapsed for a test session was 28 min including the acclimation period.

Drugs and Drug Administration

Lidocaine, norepinephrine hydrochloride (NE), DL-propranolol, and rat-human corticotropin releasing factor (CRF) were obtained from Sigma (St. Louis, MO), prazosin, idazoxan and phenylephrine were obtained from RBI (Natick, MA). Lidociane, NE, idazoxan, DL-propranolol and phenylephrine were dissolved into a specific brain buffer which in 100 ml contained 0.9 g of NaCl, 4.5 ml of 0.2 M Na_2HPO_4, and 0.95 ml of 0.2 M $NaH_2PO_4 \cdot 2H_2O$. Prazosin was dissolved into a 10% propylene glycol and CRF was dissolved into an artificial cerebrospinal fluid (CSF). These solvents were used as vehicle (Veh) for control infusion. The intra-BNST infusion device was constructed as follows: A piece of 0.5 m polyethylene tubing (PE-20, Clay Adams, Sparks, MD) 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. The drug solution was filled through the injection needle and separated from the distilled water by a tiny air bubble. Drug infusion was administered to a conscious rat. Care was taken to minimize stressing the animal. The rat was gently held and the injection needles were inserted into the cannulae with the stylet removed. To facilitate diffusion of drugs, the infusion needle protruded 1.5 mm beyond the tip of the cannulae. The rat was then placed into a small cardboard container for restraining from drastic movement. Bilateral intra-BNST infusion was administered at a rate of 0.5 µl per min through a syringe pump (CMA/100, Canergie Medicin, Stockholm, Sweden). The infusion volume was 0.5 µl for each BNST. At the end of infusion, the needle stayed in the cannula for an additional min before withdrawn and the stylet was immediately replaced to prevent back flow.

Histology Verification

At the conclusion of each experiment, animals were sacrificed with an overdose of sodium pentobarbital (50 mg per rat, ip) and perfused through the heart with 0.9% saline followed by 10 % formalin. The brain was then removed, stored in formalin for at least 48 hours. The brains were sectioned (40 µm). The brain slices stained with cresyl violet. Placements of the cannulae were examined by projecting the

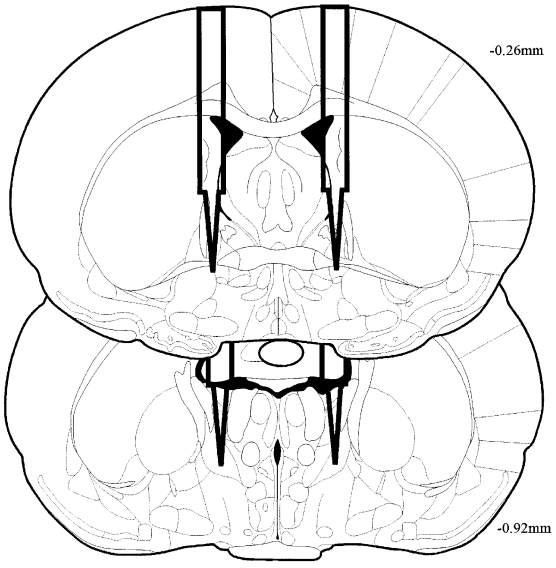

Fig. 1. Coronal plates from the atlas of Paxinos and Watson (1998) depicting the rostrocaudal extent of the target region for cannulae implanted into the bed nucleus of the stria terminalis.

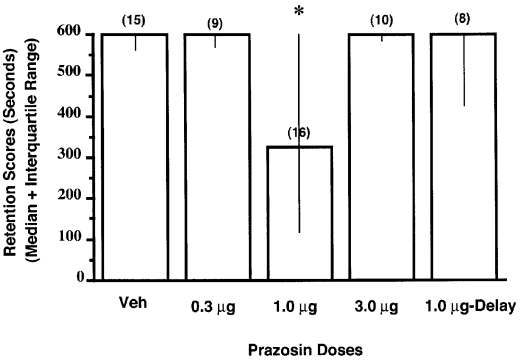

Fig. 2. Effects of posttraining intra-BNST infusion of prazosin on retention of an inhibitory avoidance task. Rats received vehicle (Veh, 10% propylene glycol) or prazosin at doses of 0.3, 1.0 or 3.0 μg immediately after training, or 1.0 μg of prazosin 4 hrs after training (Delay). * $p < 0.05$, different from the Veh group, the number in parenthesis denotes number of subjects in each group.

stained slides onto coronal plates in the brain atlas of Paxinos & Watson (48). The rostrocaudal extension of target sites is depicted in drawing of Figure 1.

Statistics

In the inhibitory avoidance task, because the distribution of retention scores was truncated at 600, this study adopted the median score to represent the central tendency of a group and the interquartile range between the 25 (Q_1) and 75 (Q_3) percentiles of the score distribution to represent the dispersion. The inhibitory avoidance data were analyzed with nonparametric statistics: Kruskal-Wallis one-way ANOVA was first used to detect differences among groups and followed by paired comparisons with the Mann-Whitney two-tailed U-tests. The scores from the locomotor activity and shock sensitivity tests were represented with means and standard errors of various groups. The data were analyzed by parametric two-way ANOVA with repeated measure designs.

Results

I. Effects of Intra-BNST Infusion of Lidocaine on Memory

Four groups of rats were trained on the task with a 1 mA/1 s footshock. Two of them received intra-BNST infusion of Veh or 2% (w/v) lidocaine immediately after training, and the remaining two received intra-BNST infusion of Veh or lidocaine 3 min before the 1-day retention test. Results showed that suppression of the BNST immediately after training caused a retention deficit: The median retention score of the Veh group was 600 s (Q_1/Q_3: 600/600, n=15), while that of the lidocaine group was 133.4 s (Q_1/Q_3: 18.9/377.1, n=13), the difference was statistically significant ($U=40$, $p<0.01$). Conversely, suppression the BNST during testing failed to affect retention performance: The median retention scores of the Veh and lidocaine groups were, respectively, 593.2 s (Q_1/Q_3: 284.5/600, n=10) and 600 s (Q_1/Q_3: 600/600, n=9), this difference was not statistically significant ($U=34.5$, $p>0.10$).

II. Effects of Intra-BNST Infusion of Prazonsin on Memory

Five groups of rats were trained on the task with a 1 mA/1 s footshock. They received intra-BNST infusion of Veh or prazosin—a α_1 antagonist—at a dose of 0.3, 1.0 or 3.0 μg immediately after training or 1.0 μg prazosin 4 hrs after training. Performance in the 1-day retention test is shown in Figure 2. A Kruskal-Wallis one-way ANOVA revealed a significant difference among the groups ($H'(4)= 10.95$, $p<0.05$). The effect was mainly due to significantly poorer retention scores of rats receiving 1.0 μg prazosin immediately after training than those of the controls ($U=64$, $p<0.05$). Conversely, rats receiving the drug 4 hrs after training had retention scores not significantly different from those of the Veh controls ($U=51.5$, $p>0.1$). While the difference between the immediate and delay infusion groups having 1.0 μg prazosin was not significant ($U=45$, $p> 0.10$), the former group tended to have more rats than the latter showing scores below the pooled median (10 vs. 2), the median test showed that difference in the two distributions approached statistical significance ($\chi^2=3.0$, $0.05<p<0.10$).

Table 1. Retention Scores (Seconds) of Rats Receiving Posttraining Infusion of Idazoxan or Propranolol

Treatments	n	Median	Interquartile Range	p
Vehicle	27	334.8	600.0-105.0	
0.1 μg idazoxan	8	361.5	600.0-153.3	ns
1.0 μg idazoxan	13	204.4	394.8- 93.0	ns
10.0 μg idazoxan	8	449.5	600.0-145.1	ns
5.0 μg propranolol	9	373.6	600.0- 30.1	ns

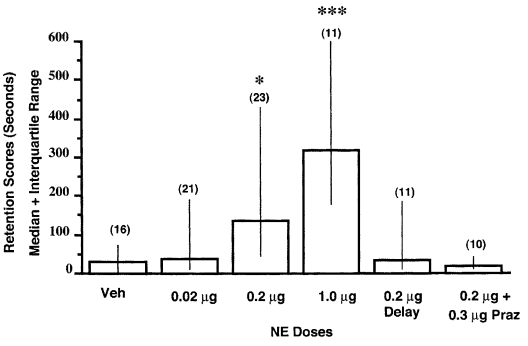

Fig. 3. Effects of posttraining intra-BNST infusion of norepinephrine (NE) on 1-day retention of the inhibitory avoidance task. Various groups of rats received vehicle (Veh, specific brain buffer), 0.02, 0.2, 1.0 μg of NE, 0.2 μg NE plus 0.3 μg prazosin (Praz) immediately after training, or 0.2 μg of NE 4 hrs after training (Delay). ***p < 0.001, *p < 0.05 different from the Veh group.

Two additional groups of rats were trained on the task without receiving any treatment in training. Intra-BNST infusion of Veh or 1.0 μg prazosin was given 3 min before the 1-day retention test. The medians for the Veh and prazosin groups, respectively, were 600 s (Q_1/Q_3: 475.2/600, n=7) and 334.8 s (Q_1/Q_3: 155.4/600, n=7). While the prazosin group appeared to have lower retention scores, the difference did not reach statistical significance (U=13.5, p> 0.10).

III. Lack of Effects of Intra-BNST Infusion of Idazoxan or Propranolol on Memory

Five groups of rats were trained as described above. They received immediate posttraining intra-BNST infusion of Veh, idazoxan—a α_2 antagonist—at a dose of 0.1, 1.0 or 10 μg, or propranolol—a β antagonist—at a dose of 5.0 μg. The results, as shown in Table 1, indicated that neither idazoxan at various doses nor propranolol at 5.0 μg administered into the BNST immediately after training produced any effect. A one-way Kruskal-Wallis ANOVA detected no significant difference among various groups (H'(4) =1.92, p>0.1), no drug-treated group had retention scores significantly different from the Veh controls (all ps>0.1).

IV. Effects of Intra-BNST Infusion of NE or Phenylephrine on Memory

Five groups of rats were trained on the task with a 0.6 mA/0.6 s footshock. They received intra-BNST infusion of Veh, 0.02, 0.2, 1.0 μg of NE or 0.2 μg NE plus 0.3 μg prazosin. An additional group trained simultaneously received intra-BNST infusion of 0.2 μg NE 4 hrs after training. The 1-day retention performance is shown in Figure 3. Immediate posttraining intra-BNST infusion of NE caused a dose- and time-dependent retention enhancement, which was attenuated by prazosin. A Kruskal-Wallis one-way ANOVA revealed a significant difference among the various groups (H'(5)=20.76, p<0.001). Further paired comparisons showed that rats having 0.2 or 1.0 μg NE immediately after training showed significant better retention scores than the Veh controls (U=92, p<0.05 and U=23.5, p<0.001). Conversely, rats having NE 4 hrs after training failed to show scores significantly different from those of the Veh controls (U=62.5, p>0.10). While the difference between the immediate and delay infusion groups having 0.2 μg NE was not significant (U=85, p>0.10), the former group tended to have more rats than the latter showing scores above the pooled median (14 vs. 3), the median test revealed that difference in the two distributions approached statistical significance (χ^2= 3.36, 0.05<p<0.10). Further, rats receiving 0.2 μg NE plus 0.3 μg prazosin had retention scores not different from the Veh group (U=80, p>0.10) but significantly lower than those receiving 0.2 μg NE only (U=39, p<0.01). These data indicated that the α_1 blocker attenuated the memory enhancing effect of NE.

To pursue further whether the enhancing effect indeed involved α_1 receptors, four groups of rats received immediate posttraining intra-BNST infusion of Veh or a α_1 agonist phenylephrine at a dose of 0.2, 1.0 or 5.0 μg. The results as shown in Table 2 indicated that posttraining intra-BNST infusion of phenylephrine caused a dose-dependent enhancement of memory. A Kruskal-Wallis one-way ANOVA detected a significant difference among the various groups (H'(3)=12, p<0.01). Further paired comparisons revealed that the 1.0 or 5.0 μg phenylephrine group had significantly better retention than the Veh group (U=5 and 6, respectively; p<0.01).

To investigate the effect of these drugs on memory retrieval, three additional groups of rats

Table 2.Retention Scores (seconds) of Rats Receiving Posttraining or Pretest Infusion of Phenylephrine

Treatments	n	Median	Interquartile Range	p*
Posttraining Infusion				
Vehicle	8	21.9	65.9- 3.9	
0.2 μg phenylephrine	7	17.1	61.2- 4.7	ns
1.0 μg phenylephrine	8	167.9	519.9-104.7	< 0.01
5.0 μg phenylephrine	8	358.5	503.5-240.9	< 0.01
Pretest Infusion				
Vehicle	9	6.0	124.2- 3.2	
5.0 μg phenylephrine	9	82.1	600.0- 15.5	ns
1.0 μg norepinephrine	7	85.7	527.8- 41.7	=0.064

*different from the Vehicle group

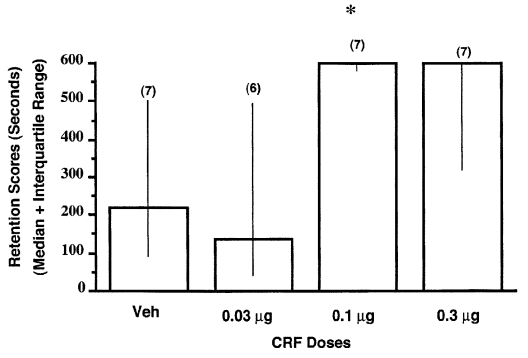

Fig. 4. Effects of posttraining intra-BNST infusion of CRF on retention of an inhibitory avoidance task. Rats received immediately after training vehicle (Veh, artificial cerebrospinal fluid) or CRF at doses of 0.03, 0.1 or 0.3 μg. * $p < 0.05$, different from the Veh group.

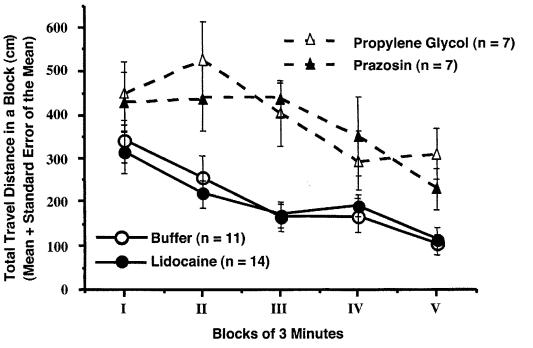

Fig. 5. Lack of effects of intra-BNST infusion of lidocaine (2%) or prazosin (1.0 μg) on a locomotor activity test. The cumulative travel distance within each of the five 3-min blocks was compared between the drug and the correspondent Veh groups.

were trained as described above but were not treated in training. They had intra-BNST infusion of Veh, 1.0 μg NE or 5.0 μg phenylephrine 3 min prior to the 1-day retention test. The results shown in Table 2 revealed that pretest intra-BNST infusion of NE or phenylephrine did not affect retention. A Kruskal-Wallis one-way ANOVA failed to find a significant difference among various groups ($H'(2)=3.94$, $p> 0.1$). While the NE and the phenylephrine groups appeared to have higher scores, paired comparisons failed to detect a significant difference ($U=14$ and 24, respectively; $p>0.05$).

V. Effects of Intra-BNST Infusion of CRF on Memory

Four groups of rats were trained on the task with a 0.7 mA/1 s footshock, and received intra-BNST infusion of Veh or 0.03, 0.1 or 0.3 μg CRF immediate after training. The 1-day retention performance is shown in Figure 4. A Kruskal-Wallis one-way ANOVA showed that difference among various groups only approached statistical significance ($H'(3)=7.5$, $p<0.06$). However, paired comparisons showed that the 0.1 μg group had significantly higher retention scores than the Veh control or 0.03 μg group ($U=7$, $p <0.05$ for both comparisons). While the 0.3 μg group also showed a high median retention score, it did not differ statistically from the Veh group.

VI. Effects of Intra-BNST Infusion of Various Drugs on Locomotion or Shock Sensitivity

Four groups were used in this experiment: Two of them received intra-BNST infusion of 2% lidocaine or specific brain buffer as vehicle, while the other two received intra-BNST infusion of 1.0 μg prazosin or 10% propylene glycol as vehicle. The results shown in Figure 5 revealed a gradually descending trend of the travel distance (in centimeters) in locomotion across successive 3-min blocks, suggesting habituation of exploration over the testing period. Neither lidocaine nor prazosin infused into the BNST produced any additional effect. These data were analyzed by two 2×5 two-way ANOVAs with Drug as a between-

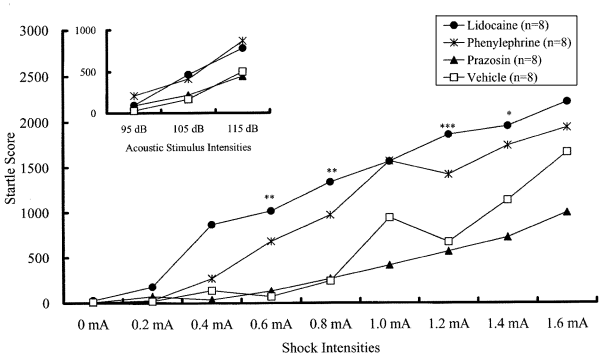

Fig. 6. Effects of intra-BNST infusion of lidocaine (2%), phenylephrine (5.0 μg) or prazosin (1.0 μg) on shock and acoustic startle. Rats received infusion shortly before startle testing. For the vehicle group, half of the subjects received propylene glycol, while the other half received specific brain buffer. * $p<0.05$, ** $p<0.01$, ***$p<0.001$, different from the vehicle group.

subject variable and Block as a within-subject variable. In both analyses, the Block main effect was significant ($F(4, 92)=16.34$, $p<0.001$ for lidocaine, $F(4, 48)=4.74$, $p<0.01$ for prazosin), the Drug main effect and Drug×Block interaction effect were not statistically significant (all $Fs<1$).

Four additional groups were tested on the shock startle task and received infusion of Veh (phosphate buffer or propylene glycol), 2% lidocaine, 1.0 μg prazosin or 5.0 μg phenylephrine infused into the BNST shortly before the test. The results, as shown in Figure 6, indicated that shock startle, but not acoustic startle, was enhanced by lidocaine suppression of the BNST; the other drugs produced no significant effects on either types of startle. These two sets of data were analyzed by two-way ANOVA with Drug as a between-subject variable and Intensity as a within-subject variable. For shock startle, the Intensity main effect was significant ($F(8, 224)=32.26$, $p<0.001$), so were the Drug main effect ($F(3, 28)=3.26$, $p<0.05$) and Drug×Intensity interaction effect ($F(24, 224)=1.64$, $p< 0.05$). Post-hoc analyses indicated that the lidocaine group showed higher startle to shocks from 0.4 to 1.4 mA ($p<0.05$) in comparison with the Veh group. For acoustic startle, the Intensity main effect was significant ($F(2, 56)=21.66$, $p<0.001$), but the Drug main effect and Drug×Intensity interaction effect were not (all $Fs<1$).

Discussion

This study reported the following findings on an inhibitory avoidance task: First, suppression of the BNST with lidocaine immediately after training impaired later retention. Second, immediate posttraining intra-BNST infusion of NE or a α_1 agonist enhanced retention, while infusion of a α_1 antagonist had an opposite effect. Drugs blocking α_2 or β adrenergic receptors had little effect at the doses tested. Third, immediate posttraining intra-BNST infusion of CRF enhanced retention. Fourth, various drugs applied to the BNST during testing failed to alter expression of the inhibitory avoidance response. These findings suggest that in the BNST, NE working through α_1 receptors and CRF are involved in modulating memory formation processes for affective experience.

Altered performance in acquisition or retention could be due to changes in sensory/motor or emotion/motivation factors rather than learning and memory per se (43). The posttraining manipulation regimen, as this study adopted, has been widely used to avoid such confounding (40), although one could still argue that sensory or emotional processing of a stimulus may continue after termination of its physical presence. The present results showed that intra-BNST infusion of lidocaine and prazosin did not affect locomotor activity. Lidocaine given to the BNST enhanced shock startle but not acoustic startle, suggesting that rats with their BNST suppressed indeed were more sensitive to shocks. A previous study also showed that ST lesions lowered the flinch-jump thresholds to electric shocks (28). The increased shock sensitivity could not account for the retention deficit caused by lidocaine infused into the BNST. Further, the present study showed that various drugs failed to affect retention when applied before testing, ruling out the possibility that rats treated as such were incapable of sensory discrimination or impaired in emotion/motivation functions. Both NE and prazosin induced a robust effect if applied to the BNST immediately after training but had no effect if applied 4 hrs later. The time-dependency of these effects argues strongly for a role of the BNST in consolidation of inhibitory avoidance memory (40).

Extensive evidence has implicated the central noradrenergic activity in memory processing (16). Consistently, the present study found that posttraining infusion of NE into the BNST, a brain region most densely innervated by NE fibers, improved formation of affective memory in a dose-dependent manner: It caused no effect at 0.02 μg but significant enhancement at 0.2 and 1.0 μg. Infusion of prazosin—a α_1 antagonist—into the same region caused an opposite effect and created a U-shaped dose-response curve: 1.0 μg impaired retention but neither lower nor higher doses had any effect. The reason that a high dose of prazosin had no effect is not readily clear. However, a previous study did report a similar shape of dose-response curve for influences of prazosin injected subcutaneously on water maze learning (51). A hypothesis that prazosin at a high dose might bind

nonspecifically to other types of receptors, e.g. presynaptic α_2 autorecptors, to counteract the effect of α_1 transmission blockade should be evaluated in the future. The enhancing and impairing effects of NE and prazosin acting on the BNST, respectively, paralleled to what has been shown by infusing these two agents into the amygdala (31).

The present study showed that prazosin at a dose of 0.3 μg blocked enhancement of memory induced by 0.2 μg of NE. This dose of prazosin had no impairing effect of its own; the attenuation thus could not be due to a pure summation of two opposite effects bearing no relevance to receptor mechanisms. These results suggest that NE infused into the BNST may act on α_1 adrenergic receptors to affect memory formation. This notion is further supported by our findings that posttraining intra-BNST infusion of the α_1 agonist phenylephrine enhanced retention. The latter effect is consistent with previous reports that α_1 agonists administered peripherally or into the amygdala enhanced memory (15, 50). On the other hand, idazoxan—a α_2 antagonist—at a wide range of doses infused into the BNST failed to cause a significant effect. Such results reduce but do not eliminate the probability of participation of BNST α_2 receptors in memory processing.

That 1.0 μg of prazosin induced a memory deficit by itself is worth noting. This finding implies that NE is released endogenously in the BNST during training and works as an intrinsic memory modulator under natural conditions. Indeed, there is evidence that stressful events such as immobilization caused in vivo NE release in the BNST (46), which played a crucial role in endocrinal and behavioral responses to a conditioned fear stimulus (44). Under the present context of addressing whether the BNST plays a role in learning and memory related to the amygdala, it is of interests to note that the stress-induced NE release in the BNST and changes of CRF immunoreactivity in the adjacent hypothalamic regions were modulated by influences from the central amygdala nuclei (3, 4).

Substantial evidence suggests that noradrenergic modulation of learning-related neural plasticity is solely β-receptor mediated, particularly in the amygdala (31, 42). However, the present study failed to find an effect of posttraining intra-BNST infusion of propranolol at the same dose (5.0 μg) that had a marked effect when given to the amygdala (31, 33). While it is viable that some other doses of propranolol may be effective, recent findings have nonetheless shown that memory modulation in the amygdala involved complicated interaction among various subtypes of adrenergic receptors. In particular, β receptors appeared to act cooperatively with α_1 receptors in altering memory formation: Pazosin infused into the basolateral amygdala nuclei attenuated the memory enhancing effect of β agonists but not that of 8-bromo-cAMP (14). In addition, concomitant infusion of a β blocker atenolol into the amygdala blocked an otherwise significant memory enhancing effect of activating α_1 receptors (15). These authors thus suggested that α_1 receptors in the basolateral amygdala modulate memory via modification of the β mediataed cAMP induction. In view of present findings that propranolol infused into the BNST did not produce a robust effect, whether a similar mode of interaction between α_1 and β receptors also prevails in the BNST is in question. However, the possibility remains that the effect of perturbing the BNST β receptors may become evident if the BNST α_1 function is concomitantly altered.

The findings that NE-containing fibers synapse with CRF immunoreactive sites in the BNST (23) suggest a possible interaction between these two neurochemicals in certain behavioral functions. A role of the hypothalamic-pituitary-adrenal axis in modulating formation of memory for affective experience has been proposed (54). The present study showed that posttraining intra-BNST infusion of CRF caused a dose-dependent enhancement of inhibitory avoidance memory with 0.1 μg being the most effective. A similar effect was also reported for infusing the same dose of CRF into the amygdala after training (27). Thus, the BNST is one of the several active sites for endogenous CRF to modulate memory. In the dentate gyrus, it has been shown that the memory enhancing effect of CRF was due to its facilitation of NE release from the presynaptic terminals, the noradrenergic modulation of memory in turn relied upon an N-methyl-D-aspartate (NMDA) mechanism (25). It is unclear whether CRF, NE and glutamate would interact in the BNST with similar mechanisms in modulating memory. Studies have shown that NE fibers in the BNST synapse on CRF containing dendrites or dendritic spines (49) and glutamate in the BNST through an NMDA mechanism enhanced release of NE (2), which in turn exerted negative feedback to inhibit glutamate release through a α_2 mechanism (18). Accordingly, NE released by glutamate in the BNST may modulate memory instead by affecting CRF release. Alternatively, CRF and NE may affect memory by their confluent action on certain intracellular biochemical events downstream to the level of their second messengers. The exact mode of interaction to affect memory processing among the various neurochemicals in the BNST would be a subject of great interests for further elucidation.

It has been proposed that the BNST mediates unconditioned but not conditioned fear responses (11). This suggestion is consistent with the report that changes in Fos or Fos-like protein induced by intra-ventricular infusion of arginine-vasopressin were

detected in the BNST of unconditioned but not conditioned rats (45). In contrast, presentation of a conditioned inhibitor readily induced expression of c-fos in the BNST (7). Further, lesions of the BNST attenuated some conditioned endocrine responses (19). Such results did suggest a correlative or even a causal role of the BNST in acquisition or expression of conditioned association. Consistent with the latter view, the present study found a significant effect on retention of an inhibitory avoidance response by suppressing the nuclei with lidocaine or perturbing its function with various adrenergic drugs or CRF shortly after training. The exact cause for the discrepant findings remains elusive. Conditioned and unconditioned fear responses might engage different neural substrates in the BNST. For example, in mice the CRF_1 and CRF_2 receptors of the lateral septum and dorsal hippocampus were shown to be differentially involved in enhancing fear conditioning acquisition and inducing anxiety (52). In view that both CRF_1 and CRF_2 receptors are found in the BNST (8), the various studies might just probe into different aspects of the BNST function due to subtle difference in their manipulating regimens and targeting regions.

Alternatively, posttraining treatments may be effective only in one-trial learning paradigms such as the inhibitory avoidance task used in the present study but not in multiple-trial paradigms such as the conditioned freezing task. However, we have shown that pre- or posttraining intra-BNST infusion of prazosin or NE affected acquisition/retention in a multi-trial and multi-session learning task-the Morris water maze (unpublished observation). A recent study showed that posttraining infusion of a $GABA_A$ agonist muscimol into the lateral/basal amygdaloid nuclei blocked memory consolidation of the inhibitory avoidance response but not that of the conditioned freezing response (61). Thus, the discrepancy could also be due to that classical conditioning is less susceptible to posttraining manipulation of BNST functions than inhibitory or active avoidance learning. In the latter type of learning, successful performance requires execution of an instrumental act in addition to association of an otherwise neutral cue with an aversive unconditioned stimulus. As a matter of fact, some previous studies have shown that the ventral amygdalofugal pathway, but not the ST, was involved in classical fear conditioning (20, 24), while our former study showed that in an inhibitory avoidance task both of these pathways were critical but subserved different roles (34).

The BNST has been proposed as part of the extended amygdala. To a certain extent, the present findings are consistent with this notion by showing that CRF and NE in the BNST were also involved in modulating formation of aversive memory, just as those in the amygdala. However, functions in learning and memory are by no means identical for these two nuclei. Our previous study has shown that suppression of the amygdala during testing impaired memory expression in the 1-day but not the 21-day test (32). In contrast, the present study showed that pretest intra-BNST infusion of lidocaine, prazosin or NE had no effect on memory expression in the 1-day test. Keeping the caution in mind that negative results are hard to interpret and effects from different studies might not share a common base for comparison, one may view these findings nonetheless implying that the modulatory role of the amygdala may sustain over a period from acquisition to expression of a recent memory, but that of the BNST could be limited to consolidation. Given the intimate reciprocal connections between the two structures, the BNST and amygdala may form with other structures a reverberatory circuitry crucial for consolidating memory traces elsewhere in the brain. Alternatively, the BNST may simply serve as a relay post for conveying the amygdala influences to other brain sites during the memory formation period. Further research addressing these two possibilities should be undertaken.

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