Pre- and Post-Training Infusion of Prazosin into the Bed Nucleus of the Stria Terminalis Impaired Acquisition and Retention in a Morris Water Maze Task

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Abstract

The bed nucleus of the stria terminalis (BNST) is interconnected with the amygdala that is implicated in memory modulation. In view of the innervation of this structure by the hippocampus and brain stem noradrenergic nuclei, this study examined the role of BNST noradrenergic activity in acquisition, formation and expression of spatial memory. Male Wistar rats with indwelling cannulae in the BNST were trained on a spatial navigation task in the Morris water maze. Groups of rats received intra-BNST infusion of vehicle, norepinephrine, prazosin or both drugs shortly before or after each daily training session, or shortly before retention tests. Results showed that pre- or posttraining infusion of 1.0 μg prazosin impaired acquisition and retention, but the treatment had no effect on a cued response task. Posttraining infusion of 1.0 μg norepinephrine enhanced acquisition and retention, and this enhancing effect was blocked by simultaneous infusion of 0.3 μg prazosin. Pretest intra-BNST of prazosin or norepinephrine at a dose of 1.0 μg did not impair expression of the spatial navigation memory. These findings suggest that the BNST noradrengergic function is involved in modulating acquisition and formation of spatial memory that engage the hippocampus.

Key Words: amygdala, hippocampus, norepinephrine, spatial memory, avoidance learning, rats

Introduction

The amygdala is implicated in memory processing of affective events. One of its memory functions is to mediate modulating influences of neurohormonal factors released by stress or emotional arousal on memory formed elsewhere in the brain (33). This function requires integrity of its major afferent-efferent pathway—the stria terminalis (ST). Early studies reported that while under certain circumstances pretraining lesions of the ST on their own impaired learning in the inhibitory avoidance task, in most cases ST lesions induced negligible effects (26). However, extensive evidence indicates that ST lesions attenuated the enhancing or impairing effects, on memory, of systemic injections of adrenal hormones (25, 44, 47), cholinergic or adrenergic drugs (17, 18) and neuropeptides (13, 34, 49). The lesion also attenuated effects of subseizure electrical stimulation of the amygdala (24) and microinfusion of norepinephrine (27) into the amygdala or oxotremorine into the caudate nucleus (38). These findings are interpreted as that the ST may convey memory modulatory influences from the amygdala to elsewhere in the brain (22).

Among the various structures innervated by the ST, the bed nucleus of the stria terminalis (BNST) receives topographical projections from the amygdaloid nuclei (9, 46) and possesses striking similarity to the amygdala in terms of cytoarchitecture, input-output connections, and neurochemical constituents (1). Functionally, the BNST is implicated in stress

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modulation of autonomic, endocrinal and somatomotor activities (15), such as being startled by acoustic noise bursts (6). The anatomical relationship between the two structures suggests that the BNST may carry out its functions by interacting with the amygdala. One of such possibilities is that the BNST mediates certain functions of the amygdala. Findings on classical fear conditioning did not favor a role of the BNST in mediating conditioned heart rate or freezing responses activated by the amygdala (21). However, a former study demonstrated that in the inhibitory avoidance task, intra-BNST infusion of naloxone attenuated the memory deficit caused by electrical amygdaloid stimulation given immediately after training (28). This evidence supports a role of the BNST in mediating the amygdala influences on aversive learning, and predicts that manipulating the BNST function shortly after training should affect acquisition or retention. This prediction was confirmed by the findings that posttraining intra-BNST infusion of levorphanol, an opiate antagonist, impaired retention of the inhibitory avoidance response (28). A recent study in this laboratory further showed that posttraining suppression of the BNST with local infusion of lidocaine impaired memory of an inhibitory avoidance response (23).

Evidence has shown that the BNST is innervated densely by the A_1 and A_2 noradrenergic cell groups (14) and contains the highest concentration of norepinephrine in the brain (2). It expresses α_1, α_2, β_1 and β_2 adrenergic receptors (7, 43, 48), as well as adrenergic transporter for reuptake (19). Aversive conditioned stimulus elevated norepinephrine turnover rate in the BNST (35). In congruence, posttraining intraBNST infusion of norepinephrine caused a memory enhancing effect and the effect was mimicked by phenylephrine and blocked by prazosin (23), suggesting potential involvement of BNST α_1 adrenergic receptors in modulating formation of memory for aversive experience.

In addition to the amygdala, the BNST is also intimately connected with the hippocampus (5, 52) and has been shown to mediate certain types of hippocampal functions such as the feedback inhibition of glucocorticoid on the hypothalamus-pituitary-adrenal activity (53). Spatial navigation in a Morris water maze is a typical task probing the hippocampal memory function. Performance in this task is affected by stress, an effect presumably mediated *via* the amygdala (20) as direct amygdala manipulation induced similar effects (37). These findings lead to an expectation that the BNST should also be involved in modulating spatial memory. To assess whether the role of BNST noradrenergic activity in inhibitory avoidance memory can be generalized to another type of aversive learning, the present study was designed to investigate the effect of pretraining, posttraining or pretest infusion of prazosin, an α_1 adrenergic antagonist, and norepinephrine into the BNST on acquisition and retention of a spatial navigation task in the Morris water maze.

Materials and Methods

Subjects

Subjects of the present study were male Wistar rats about 5 to 6 months old, weighing about 350 to 400 grams. They were individually housed in our animal facilities upon arrival from the National Breeding Center of Experimental Animals (Nankang, Taipei). Food and water were available all the time. Throughout the study a 12:12 light:dark cycle was adopted with lights on at 8:00 a.m. All experimental procedures were carried out in accordance with Guidelines for Animal Research in Ethical Codes of Chinese Psychological Association and were approved by the Committee for Use of Experimental Animal in the National Taiwan University.

Surgery

The rats received bilateral guide cannulae implantation into the BNST. They received an anesthetic dose of sodium pentobarbital (ip, 45 mg/kg); atropine sulfate (0.4 mg/kg) was given 10 min before the anesthetic to prevent respiratory congestion. The anesthetized rat was mounted on a DKI-900 stereotaxic instrument; coordinates for cannula implantation were AP. -0.2 mm, ML. ±1.7 mm and DV. ±4.0 mm with the incisor bar set at -3.3 mm. A cannula at a length of 15 mm and made of 23 G stainless steel tubing with 0.33 mm inner diameter and 0.63 mm outer diameter was implanted into the BNST. 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. The rats were kept warm until resurrection from the operation.

Morris Water Maze

Spatial navigation was trained on a Morris water maze about a month after recovery from the surgery. As described in a former study (29), it was performed in a circular plastic pool (235 cm diameter, 45 cm height) located in a room with distinctive visual cues. Water was filled to a depth of 36 cm and a transparent plastic platform (25 × 25 cm, 32 cm height) was located in a fixed quadrant 35 cm away from the nearest wall. To adapt for the maze task, rats received a trial of 2-min free swimming in the pool one day before formal training trials. Rats then received 4

training trials per day for 6 consecutive days. On each trial, the rat entered the water randomly from one of the four starting places. The rat had to navigate in the pool until climbing onto the platform. The escape latency, which was the duration from entering water to reaching the platform, was measured in each acquisition or retention trial by an observer with a timer. If the rat failed to locate the platform by 120 s, it was picked up and placed onto the platform by the experimenter and a score of 120 was assigned for that trial. The rat stayed on the platform during a 60-s inter-trial interval. In some experiments, rats received intra-BNST infusion before or after a daily training session. After six days of training, rats such treated were subject to a probe test on the 7th day in a drug-free state. In other experiments, rats received pretest treatments, after six days of drug-free training, they were subjected first to a regular 4-trial test on the 7th day and then to a probe test on the 9th day. Infusion of drugs or vehicle was given before the regular test and the probe test, but neither behavioral test nor drug treatment was administered on the 8th day.

In the probe test, the hidden platform was removed from the pool and the rat was given 2 min of free swimming in the pool. The swimming time in each quadrant was measured by a computerized event recording program capable of cumulating the duration of the rat's presence in various quadrants through stroking different computer keys by an observer.

Drugs and Drug Administration

Norepinephrine hydrochloride was obtained from Sigma (St. Louis, MO), while prazosin was obtained from RBI (Natick, MA). Norepinephrine was 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 10% propylene glycol. The solvents were used as vehicle for infusion in the control group. The dose for both prazosin and norepinephrine was 1.0 μg, which was most effective according to our previous findings (23). 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 Gauge 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 infusion 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 to restrain 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 minute before it was withdrawn and the stylet was immediately replaced to prevent back flow. Pretraining infusion was dispensed five minutes before the first training trial of each daily session; posttraining infusion was dispensed immediately after the last training trial of each training session. Pretest infusion was dispensed five min before the start of the regular 4-trial test session and also before the probe test.

Statistics

Acquisition performance was judged by escape latencies in the 4 daily trials over the 6 training days. These data were analyzed by 3-way ANOVAs of mixed design with Drug as a between subject variable, and Day and Trial as the within-subject variables. Retention performance in a regular test was also judged by the escape latency, the data were analyzed by 2-way ANOVAs of mixed design with Drug as a between-subject variable and Trial as a within-subject variable. Post-hoc Scheffe tests were used for multiple comparisons on any possible pair of means. Retention performance in a probe test was represented by the mean searching time per quadrant, which was defined as the average time of swimming in a single quadrant. For the target quadrant, this index just equaled the swimming time within it. For the non-target quadrants, it was calculated as the total swimming time in all three of them divided by 3. The data construed as such comply with the linear independence assumption of ANOVA and thus can be subjected to two-way ANOVAs of mixed design with Drug as the between-subject variable and Quadrant as the within-subject variable. Post-hoc Newman-Kuel tests were used to detect difference in the mean searching time between the target and non-target quadrants under different drug treatments.

Histology Verification

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

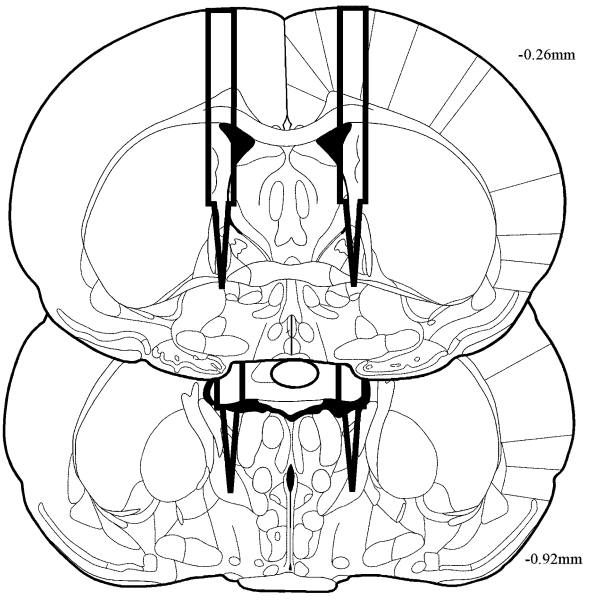

Fig. 1. Coronal plates from the atlas of Paxinos and Watson (1998) depicting schematically the rostrocaudal extent of cannulae placement rostrocaudal (Adapted by permission).

slides onto coronal plates in the brain atlas by Paxinos & Watson (40). Fig. 1 shows the placement of cannulae placement schematically.

Results

Pretraining Intra-BNST Infusion of Prazosin Impaired Acquisition and Retention of the Spatial Response

Two groups of rats received intra-BNST infusion of vehicle or 1.0 μg prazosin shortly before training. The left panel of Fig. 2 shows the acquisition curve, represented by the mean escape latency of 4 training trials on each day, for different groups over 6 days of training. As shown in the figure, pretraining infusion of prazosin into the BNST impaired acquisition in the water maze task. A three-way ANOVA showed that pretraining infusion of 1.0 μg prazosin increased the escape latency as indicated by a significant Drug main effect ($F(1, 14) = 8.11$, $P < .001$). Nonetheless, both groups showed improvement within each daily session and across the 6 training days, as both Trial and Day main effects were statistically significant ($F(3, 42) = 13.25$, $P < .001$; $F(5, 70) = 22.70$, $P < .001$), no interaction effect was statistically significant.

After six days of training, the two groups received a probe test under a drug-free state. The mean searching times per quadrant for the target and non-target ones are shown in the right panel of Fig. 2. The vehicle group spent more time of searching in the target quadrant than in a non-target one, but the prazosin group did not. A two-way ANOVA showed that both the Drug main effect and Drug×Quadrant interaction effect were statistically significant ($F(1, 14) = 9.36$, $P < .01$; $F(1, 14) = 12.33$, $P < .01$, respectively), but the Quadrant main effect did not ($F(1, 14) = 2.54$, $P > .10$). Post-hoc tests showed that the mean searching time per quadrant for the target and non-target ones differed in the vehicle group ($P < .01$) but not in the prazosin group.

Posttraining Intra-BNST Infusion of Prazosin Impaired Acquisition and Retention of the Spatial Response

Two groups of rats received intra-BNST infusion of vehicle or 1.0 μg prazosin shortly after training. Results shown in the left panel of Fig. 3 indicate that posttraining infusion of prazosin into the BNST impaired acquisition in the water maze task. Rats having prazosin in the BNST immediately after the daily training session took longer time to reach the platform than the controls, as indicated by a significant Drug main effect ($F(1, 12) = 13.17$, $P < .01$). Yet both groups improved across successive training sessions as indicated by a significant Day main effect ($F(5, 60) = 8.94$, $P < .05$). The Trial main effect only approached statistical significance ($F(3, 36) = 2.45$, $.07 < P < .08$). All interactions were not significant ($Fs \leq 1$ for all comparisons).

After six days of training, the two groups received a probe test under a drug-free state. The mean searching times per quadrant for the target and non-target ones are shown in the right panel of Fig. 3. The vehicle group, but not the prazosin group, spent more time of searching in the target quadrant than in a non-target one. The Drug main effect and Drug× Quadrant interaction were statistically significant ($F(1, 12) = 9.57$, $P < .01$; $F(1, 12) = 8.17$, $P < .05$, respectively), but the Quadrant main effect failed to reach the level of significance ($F(1, 12) = 3.67$, $P = .08$). Post-hoc tests showed that difference in the mean searching time per quadrant between target and non-target ones was statistically significant in the vehicle group ($P < .05$) but not in the prazosin group.

Pretest Intra-BNST Infusion of Prazosin had no Effect on Expression of the Spatial Memory

Two groups of rats later to be treated before testing received no treatment during the acquisition phase. As indicated in the left panel of Fig. 4, no inherent difference for acquiring the task was detected in the two groups. They made almost identical progress in each daily session and across 6 days, as revealed by a non-significant Group main effect ($F(1, 17) < 1$), as well as significant Trial and Day main effects ($F(3, 51) = 7.40$, $P < .001$; $F(5, 85) = 21.70$, $P < .001$;

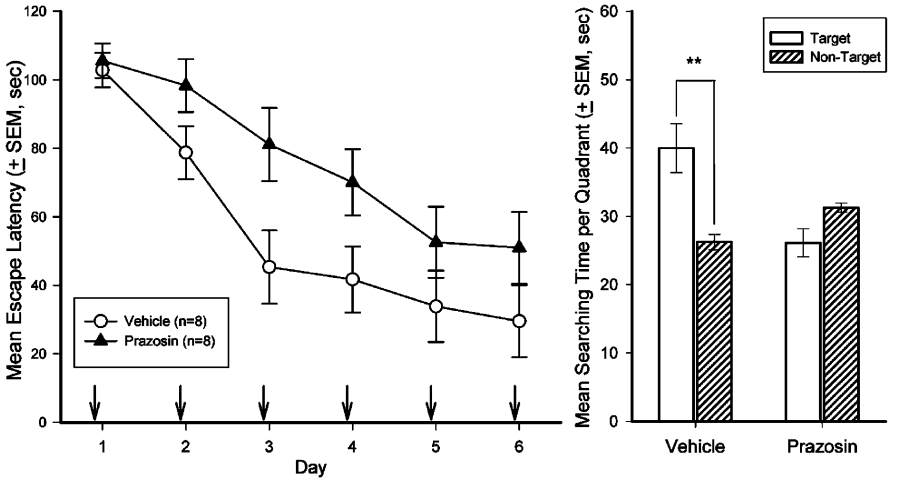

Fig. 2. Effects of pretraining intra-BNST infusion of 1.0 μg prazosin on acquisition during the training sessions (left panel) and retention in the probe test (right panel). Arrows on the x-axis indicate that prazosin or vehicle was administered into the BNST just prior to each daily training session. The escape latency for each daily session was the average of escape latencies for the 4 training trials on that day. The mean searching time per quadrant was calculated as the total swimming time in the target or non-target quadrants divided by the number of quadrants in that category (1 or 3 in the present study, respectively). $^{**}P < .01$.

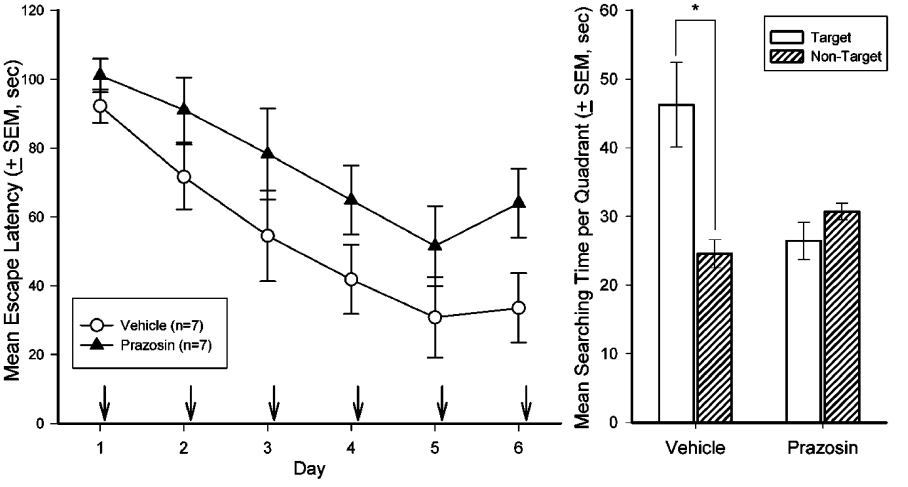

Fig. 3. Effects of posttraining intra-BNST infusion of 1.0 μg prazosin on acquisition in the training sessions (left panel) and retention in the probe test (right panel). Arrows on the x-axis indicate that prazosin or vehicle was administered into the BNST immediately after each daily training session. $^{*}P < .05$.

respectively). No interaction effect was statistically significant (all $Fs < 1$).

After six days of training, the two groups were subjected to a regular 4-trial test on the 7th day and a probe test on the 9th day. They received intra-BNST infusion of vehicle or 1.0 μg of prazosin shortly before both tests. Analysis of the regular test data (the middle panel of Fig. 3) revealed that the Trial main effect was statistically significant ($F(3, 51) = 3.21$, $P < .05$), but neither the Drug main effect nor the Drug×Trial interaction effect was significant (both $Fs < 1$).

Data of the probe test are shown in the right panel of Fig. 4. Both groups of rats preferred the target quadrant by having longer mean searching time per quadrant in it. The Quadrant main effect was statistically significant ($F(1, 17) = 28.57$, $P < .001$), and the Drug main effect and Drug×Quadrant interaction effect approached statistical significance ($F(1, 17) = 3.93$, $P = .06$; $F(1, 17) = 3.93$, $P = .06$, respectively). Post-hoc tests showed that the difference in the mean searching time per quadrant between the target and non-target ones was significant in both the vehicle group ($P < .001$) and the prazosin group ($P < .05$).

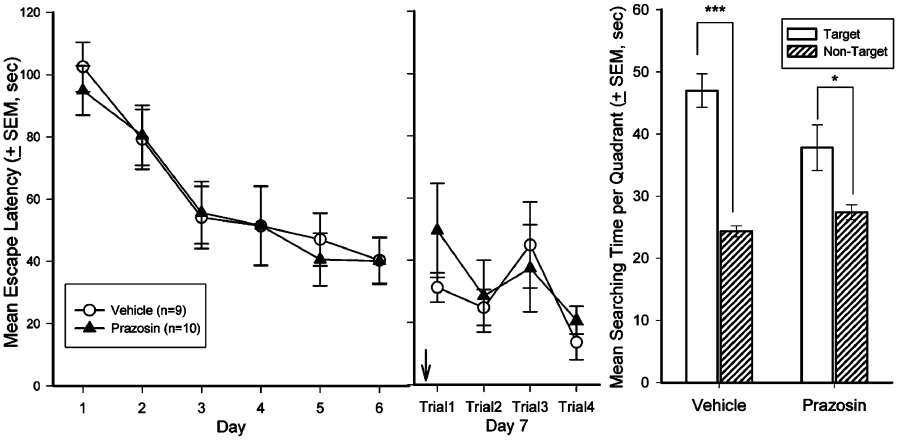

Fig. 4. Lack of effect of pretest intra-BNST infusion of 1.0 μg prazosin on retention in a regular test and a probe test. Two groups of rats matched on acquisition (left panel) had prazosin or vehicle infused into the BNST just prior to a regular test (middle panel, arrows on the x-axis marks the infusion) and prior to the probe test (right panel). ***$P < .001$, *$P < .05$.

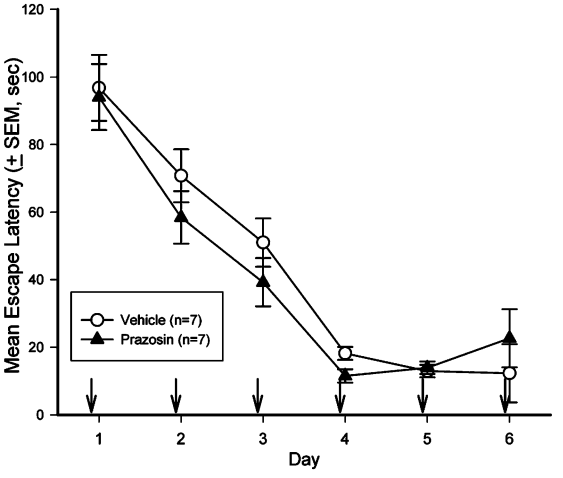

Fig. 5. Lack of effect of pretraining intra-BNST infusion of 1.0 μg prazosin on acquisition of a visual discrimination response in the training sessions. Arrows on the x-axis indicate that prazosin or vehicle was infused into the BNST just prior to each daily training session.

Pretraining Intra-BNST Infusion of Prazosin did not Affect Cued Response Learning

Two groups of rats received pretraining intra-BNST infusion of vehicle or 1.0 μg prazosin and were subjected to a cued response task in the Morris water maze, in which the platform was raised above the water level and marked with distinctive cues. Performance during training is shown in Fig. 5. Data analysis revealed significant Trial and Day main effects ($F(3, 36) = 5.90$, $P < .01$; $F(5, 60) = 58.96$, $P < .001$; respectively). Other effects were insignificant (all $Fs < 1$). Thus, the two groups acquired the visual discrimination task equally well.

Posttraining Intra-BNST Infusion of Norepinephrine Enhanced Acquisition and Retention of the Spatial Response

Three groups of rats subjected to the task were treated with intra-BNST infusion of vehicle, 1.0 μg norepinephrine or 1.0 μg norepinephrine plus 0.3 μg prazosin immediately after each training session. The results in the left panel of Fig. 6 indicate that rats with posttraining intra-BNST infusion of norepinephrine appeared to have shorter escape latencies than others. Analysis of the data revealed a significant Drug main effect ($F(2, 28) = 3.51$, $P < .05$). Multiple comparisons among the three treated groups by Scheffe tests indicate that the norepinephrine group significantly differed from the vehicle group in mean escape latencies ($P < .05$). Yet all three groups showed improvement across trials within a session and over the 6 training days as the Trial and Day main effects were all significant ($F(3, 84) = 14.29$, $P < .001$; $F(5, 140) = 48.65$, $P < .001$). No two-way interaction was significant. The Drug×Day×Trial three way interaction effect approached statistical significance ($F(30, 420) = 1.47$, $P < .06$).

The mean searching times per quadrant for the target and non-target ones in the probe test are shown in the right panel of Fig. 6. Rats having norepinephrine after training spent more time in searching the target quadrant. The data were analyzed by a two-way mixed design ANOVA as described previously. The Quadrant main effect was statistically significant ($F(1, 28) = 17.79$, $P < .001$), but the Drug main effect and Drug×Quadrant interaction did not (both $Fs < 1$). Post-hoc tests showed that the difference in the mean searching time per quadrant between the target and non-target ones was significant in the norepinephrine group ($P < .05$) but not in the other two groups.

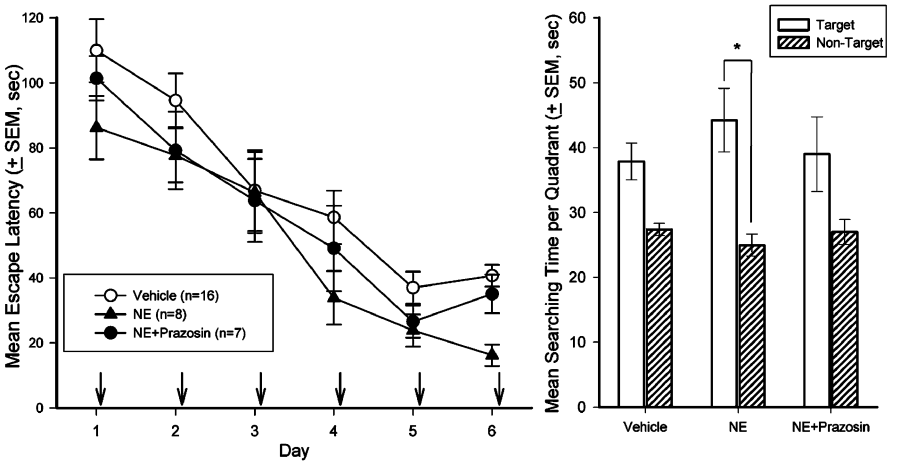

Fig. 6. Effects of posttraining intra-BNST infusion of 1.0 μg norepinephrine (NE) or 0.3 μg prazosin plus 1.0 μg norepinephrine on acquisition in the training sessions and on retention in the probe test. Arrows on the x-axis indicate that prazosin and/or norepinephrine was administered into the BNST immediately after each daily training session. *$P < .05$.

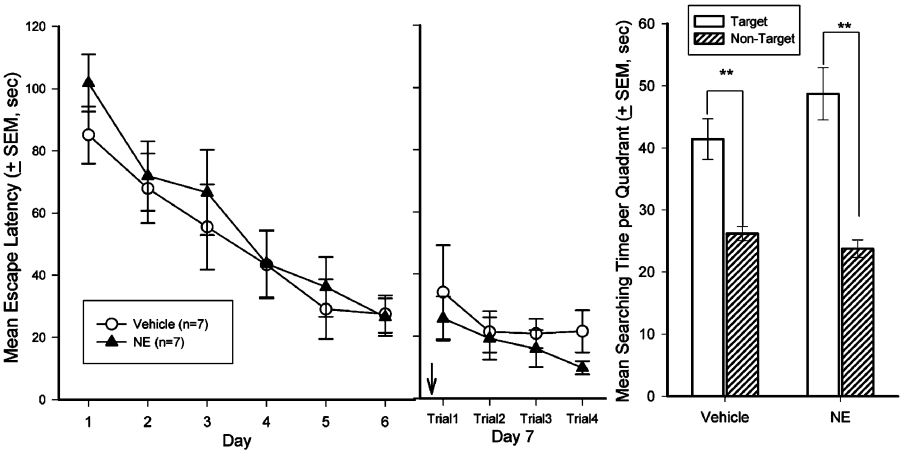

Fig. 7. Lack of effect of pretest intra-BNST infusion of 1.0 μg norepinephrine (NE) on retention in a regular test and a probe test. Two groups of rats matched on acquisition (left panel) had norepinephrine or vehicle infused into the BNST just prior to a regular test (middle panel, arrows on the x-axis marks the infusion) and prior to the probe test (right panel). ** $P < .01$.

Pretest Intra-BNST Infusion of Norepinephrine did not Affect Expression of Spatial Memory

Two groups of rats later to be treated before the retention test received no drug treatment during the acquisition phase. Their acquisition performance was shown in the left panel of Fig. 7. Analysis of the data revealed that the two groups made almost identical progress in each daily session and across the 6-day training period, as revealed by a non-significant Group main effect ($F(1, 12) < 1$) but significant Trial and Day main effects ($F(3, 36) = 12.86$, $P < .001$; $F(5, 60) = 29.97$, $P < .001$; respectively). No interac-tion effect was statistically significant (all $Fs < 1$).

After six days of training, the two groups received the regular and probe tests as described previously. Norepinephrine or vehicle was administered before each test. Analysis of the regular test data (the middle panel of Fig. 7) showed that neither the Drug main effect nor the Trial main effect was statistically significant ($F(1, 12) = 1.24$; $F(3, 36) = 1.40$, $P > .10$). Mean searching times per quadrant in the probe test on the 9th day are shown in the right panel of Fig. 7. Both groups of rats spent more time of searching in a target quadrant. Analysis of the data revealed that the Quadrant main effect was statistically significant ($F(1, 12) = 32.04$, $P < .001$), but the Drug main effect was not ($F(1, 12) = 1.87$, $P > .10$). Posthoc tests showed that difference in the mean searching times per quadrant between the target and non-target ones was significant both in the vehicle group ($P < .01$) and the norepinephrine group ($P < .01$). The

magnitude of preference did not differ between the two groups as the Drug×Quadrant interaction effect was not significant ($F(1, 12) = 1.87$, $P > .10$).

Discussion

Results of the present study show that in a Morris water maze, pre- or post-training infusion of prazosin into the BNST impaired acquisition and/or retention of a spatial navigation response, whereas infusion of norepinephrine into the same region improved acquisition and/or retention, and this enhancement was attenuated by simultaneous infusion of prazosin. Conversely, neither norepinephrine nor prazosin infused into the BNST before a retention test affected expression of well-established memory. These findings suggest that noradrenergic activity in the BNST is involved in acquisition or formation, but not retrieval, of spatial memory in a Morris water maze.

Treatments applied before a training trial could alter perceptual, motivational, or motor abilities, and thus influence performance rather than learning or memory processes per se (31). However, this account is implausible for the present effects. First, in a probe session in which the rats were tested under a drug-free state, the group treated with prazosin during training showed persistent deficits by expressing weak preference for the target quadrant. Second, the same treatment given before testing did not affect performance. Third, prazosin given after training could not possibly affect performance during training, yet it impaired memory as well. Fourth, prazosin did not affect cued response learning in the water maze, which demands similar perceptual, motor, and motivational abilities as the spatial learning task. The last finding also argues for a specific effect of the drug on spatial memory relative to cued response memory. These two types of learning have been shown to depend upon different brain systems (30); it is thus conceivable that acquisition, memory formation and retrieval in these two tasks involve distinct neural processes that have differential susceptibility to prazosin.

In the Morris water maze, subjects reach the asymptotic performance after several days of multiple-trial training; a pretraining treatment thus could affect the rate of learning by acting on acquisition, consolidation or both. The present study showed that intra-BNST infusion of prazosin immediately after training resulted in a memory deficit similar in extent as that resulted from the pretraining treatment. Thus, a detrimental effect of prazosin on consolidation of spatial memory clearly contributes to the amnesia observed in the present study. However, prazosin did impair acquisition as suggested by a significant Trial main effect in the pretraining infusion experiment but not in the posttraining infusion experiment. Thus, the drug infused before training retarded acquisition in a daily session as well as consolidation afterwards.

Consistently, posttraining infusion of norepinephrine enhanced retention: In the training phase, the norepinephrine group acquired the response better than the controls; in the probe test, it also showed stronger preference for the target quadrant comparing with the controls. These findings are congruent with a view that the BNST is a site, among others, for norepinephrine to modulate memory. Such a view is consistent with an abundance of noradrenergic terminals and receptors (50) as well as *in vivo* release of norepinephrine during immobilization stress in this structure (36). The present results expand the memory-modulating role of BNST noradrenergic activity from an inhibitory avoidance task (23) to a task dependent on the hippocampus, and thus supports a function of BNST norepinephrine in modulating general neuroendocrine and behavioral reactions under stress (35). However, the effect of norepinephrine in this study, while statistically reliable, was relatively mild. Future studies should explore a wider dose range of norepinephrine and more suitable training conditions to demonstrate robust memory enhancement in spatial tasks.

Our data also indicate that prazosin at a non-impairing dose (23) attenuated the memory enhancing effect of norepinephrine. These results suggest, yet do not prove, a potential role of α_1 noradrenergic receptors located postsynaptically in the BNST (7, 48) in modulating spatial memory. This suggestion is congruent with a notion that noradrenergic modulation of memory in various tasks involves not only β but also α_1 receptors (11, 12). It follows that α_1 noradrenergic agonists, such as phenylephrine, infused into the BNST shortly after training should facilitate memory. This prediction has been confirmed in an inhibitory avoidance task (23) and ought to be further tested in the Morris water maze task. However, it should be noted that formation of spatial memory is not solely dependent upon the BNST α_1 noradrenergic activation, because rats treated with prazosin still made significant, even though slower, progress over the training period. Involvement of other adrenergic receptor subtypes, such as α_2, β_1, or β_2, of the BNST in spatial memory ought to be studied in the future.

A previous study has shown that elicitation or suppression of conditioned fear altered *c-fos* expression in the BNST (3). Such evidence raises a possibility that the BNST might be responsible for expressing certain forms of conditioned fear responses, a notion consistent with the findings that lesions of the BNST hampered release of corticosteroid elicited by conditioned fear stimuli (16). However, lesions before conditioning could have affected acquisition, formation or retrieval processes and thus the reported effect might not be specific to memory expression per

se. By manipulating the BNST at distinct phases of memory processing, the present study showed that the BNST noradrenergic activity did not play a role in expression of memory, at least at the dose tested and in the Morris water maze, consistent with findings in the inhibitory avoidance task (23). It is possible that higher doses of prazosin may be effective or other subsystems in the BNST may subserve the function. However, our recent findings showed that total suppression of the BNST with lidocaine during testing also failed to affect spatial memory expression (Chen & Liang, in preparation), which rendered this possibility less likely. Thus, the BNST activation induced by a conditioned stimulus may be merely a non-causal correlate of the conditioned behavior. On the other hand, the present findings are consistent with a role of the BNST in mediating unconditioned fear (51), such a role allows the nuclei to be activated by stressful events during the training phase and hence modulate the formation of aversive memory.

How the BNST modulates various types of memory invites speculation. As previously noted, the BNST is involved in mediating both the physiological and behavioral effects of stress (15). Stress modulates the hippocampal memory system (8). Acquisition and expression of spatial memory are subjected to influences of stress and this effect may be mediated by the amygdala (20). Previous studies have shown that posttraining intra-amygdala infusion of amphetamine enhanced retention of either a spatial response or a cued response in a Morris water maze depending on the hippocampus or caudate nucleus, respectively (37, 39). The findings suggest that stress working through the amygdala can act on the hippocampus or caudate nucleus to differentially modulate two distinct memory systems. Such modulation may somehow involve the BNST. Consistent with this conjecture are the findings that lesions of the stria terminalis attenuated the memory effect of glucocorticoid infused into the caudate nucleus (44).

The neural mechanism by which the BNST interacts with the hippocampus remains to be elucidated. The BNST receives hippocampal inputs (5, 52), and spatial information might be transmitted to the BNST and thereby modified by noradrenergic inputs. The results that the BNST played no role in expressing spatial memory suggest that spatial processing is not likely to encounter noradrenergic modulation directly at the BNST. On the other hand, given the role of BNST in regulating stress release of corticosteroid (10, 16) and the importance of this hormone in modulating hippocampal functions (45), perturbation of the BNST noradrenergic system may affect the hippocampus memory system *via* altering the hypothalamus-pituitary-adrenal axis (15). Consistent with this speculation are the findings that blocking α_1 noradrenergic receptors in the BNST attenuated the corticosteroid release as well as the anxiety behavior in an elevated plus maze (4). Another possibility is that altering the BNST noradrenergic function may reciprocally affect activity in the amygdala through connections between the two structures, and hence alter the hippocampal memory function *via* direct or indirect amygdaloid projections to the hippocampus (41, 42), as suggested by a recent proposal (32). The exact nature of this action awaits further elucidation.

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