Neurobiology of Learning and Memory 91 (2009) 456–465

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Post-training infusion of glutamate into the bed nucleus of the stria terminalis enhanced inhibitory avoidance memory: An effect involving norepinephrine

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ARTICLE INFO

Article history:
Received 20 October 2008
Revised 10 January 2009
Accepted 12 January 2009
Available online 30 January 2009

Keywords:
Affective memory
Memory consolidation
NMDA receptor
Adrenoceptor
Amygdala
Rats

ABSTRACT

This study examined an interaction between glutamate and norepinephrine in the bed nucleus of the stria terminalis (BNST) in modulating affective memory formation. Male Wistar rats with indwelling cannulae in the BNST were trained on a one-trial step-through inhibitory avoidance task and received pre- or post-training intra-BNST infusion of glutamate, norepinephrine or their antagonists. Results of the 1-day test indicated that post-training intra-BNST infusion of DL-2-amino-5-phosphonovaleric acid (APV) impaired retention in a dose- and time-dependent manner, while infusion of glutamate had an opposite effect. Co-infusion of 0.2 μg glutamate and 0.02 μg norepinephrine resulted in marked retention enhancement by summating non-apparent effects of the two drugs given at a sub-enhancing dose. The amnesic effect of 5.0 μg APV was ameliorated by 0.02 μg norepinephrine, while the memory enhancing effect of 1.0 μg glutamate was attenuated by 5.0 μg propranolol. These findings suggest that training on an inhibitory avoidance task may alter glutamate neurotransmission, which by activating NMDA receptors releases norepinephrine to modulate memory formation via β adrenoceptors in the BNST.

1. Introduction

The amygdala is implicated in processing affective memory, and such a function may involve a major amygdala pathway—the stria terminalis (ST). Pre-training ST lesions blocked the amnesic effect of electrical amygdala stimulation on the inhibitory and active avoidance tasks (Liang & McGaugh, 1983a) and the memory modulatory actions of various central or peripheral treatments mediated by the amygdala in these tasks (Liang & McGaugh, 1983b; McGaugh, Introini-Collison, Juler, & Izquierdo, 1986; Roozendaal & McGaugh, 1996; Torras-Garcia, Costa-Miserachs, Portell-Cortes, & Morgado-Bernal, 1998). Because the amnesic effect of electrical amygdala stimulation was also attenuated by naloxone infused into the bed nucleus of the ST (BNST) (Liang, Messing, & McGaugh, 1983)—a structure innervated by the ST met-enkaphalin fibers arising from the central amygdala nuclei (Uhl, Kuhar, & Synder, 1978), the ST efferent was proposed to mediate the amygdala influences on memory (Liang, McGaugh, & Yao, 1990).

Such findings hinted a role of the amygdala efferent target BNST in memory processing. An early study found that in fear conditioning the BNST mediated a conditioned endocrine response (Gray et al., 1993). Later studies showed that by acting on the BNST, corticotrophin releasing hormone (CRH) enhanced inhibitory avoidance memory (Liang, Chen, & Chen, 2001) and its non-selective antagonist alpha-helical CRH_{9-41} blocked conditioned freezing (Nijsen, Croiset, Diamant, De Wied, & Wiegant, 2001). A recent study showed that the BNST was essential for expression of conditioned behavioral and hormonal responses in contextual fear conditioning (Sullivan et al., 2004). Neural correlates of memory are also detected in the BNST: Expression of c-*fos* in the BNST was altered by classical fear conditioning (Campeau et al., 1997) or by activating memory of neuroendocrine reactions to vaginocervical stimulation (Polston, Heitz, Barnes, Cardamone, & Erskine, 2001). Drug or natural reward received via acquired operant behavior, but not via passive delivery, enhanced excitatory synaptic transmission in the BNST (Dumont, Mark, Mader, & Williams, 2005).

Consistent with the high concentration of norepinephrine (Phelix, Liposits, & Paull, 1992) and abundance of adrenoceptors in the BNST (Day, Campeau, Watson, & Akil, 1997; Pieribone, Nicholas, Dagerlind, & Hokfelt, 1994; Rainbow, Parsons, & Wolfe, 1984), previous studies showed that manipulation of BNST noradrenergic activity affected retention in an inhibitory avoidance task (Liang et al., 2001), Morris water maze (Chen, Chen, Chen, & Liang, 2004) and conditioned fear-potentiation of startle (Schweimer, Fendt, & Schnitzler, 2005). In the ventrolateral portion of the BNST, dense NMDA-NR1 receptors are presented on the terminals of axons (Gracy & Pickel, 1995) that arise, in part, from the A1 or A2 noradrenergic neurons (Forray, Gysling, Andres, Bustos, & Araneda, 2000). Superfusion of NMDA to brain slices containing the ventral

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doi:10.1016/j.nlm.2009.01.003

BNST increased norepinephrine release (Forray, Andres, Bustos, & Gysling, 1995), suggesting that glutamate acting in the BNST may cause *in vivo* release of norepinephrine that has been implicated as an endogenous memory modulator.

The aforementioned evidence predicts that the BNST glutamate could play a role in memory processing. Previous evidence showed that acquisition of an operant act altered the ratio of currents mediated by different glutamate receptor subtypes in the BNST (Dumont et al., 2005). However, a causal role of the BNST glutamatergic system in memory processing remains to be tested. This study thus examined the effect of post-training intra-BNST infusion of glutamate or NMDA antagonists on retention of an inhibitory avoidance response; and if such an effect was indeed observed, whether it was related to an action of norepinephrine.

2. Materials and methods

2.1. Subjects

Male Wistar rats weighing from 300 to 350 g were used in this study. Upon arriving from National Breeding Center of Experimental Animals, they were housed individually in our animal facilities under a 12:12 h light:dark cycle (lights on at 7:00 am) and had free access to food and water. The room temperature was maintained at 22 ± 2 °C with humidity at 60–70%. Animal care and research procedure adhered to Guidelines for Animal Research of Agriculture Council, ROC and Ethical Codes of Taiwanese Psychological Association; all experimental protocols were approved by Institutional Animal Care and Use Committee at National Taiwan University.

2.2. Brain surgery

Rats received stereotaxic surgery to implant guide cannulae bilaterally into the BNST. They were anesthetized with sodium pentobarbital (50 mg/kg, ip) following atropine sulfate (0.5 mg/kg, ip) to prevent respiratory congestion. The anesthetized rat was mounted on a stereotaxic instrument (David Kopf Instruments, DKI-900, Tujunga, CA, USA). The skull was exposed and burr holes were drilled over the BNST target. Two 15-mm guide cannulae were inserted into the BNST at the coordinates of AP +1.4 mm, ML ±1.0 mm, and DV −5.5 mm with the nose bar set at +5.0 mm relative to the inter-aural line (Pellegrino, Pellegrino, & Cushman, 1979). The cannulae were made of 23-Gauge stainless steel tubes with 0.63 mm outer diameter and 0.33 mm inner diameter. Three jewelry screws were implanted on the skull as anchors. The whole complex was affixed on the skull with dental cement. A stylet at a length of 16 mm was inserted into each cannula to maintain patency. After surgery the rats were allowed to recover for 10 days. Then they received daily checking and handling for 5 days before behavioral training and testing.

2.3. Drug and brain infusion

Drugs including DL-2-amino-5-phosphonovaleric acid (APV), (-)-norepinephrine, and DL-propranolol were obtained from Sigma (St. Louis, MO, USA), while prazosin and L-glutamate were obtained from RBI (Natick, MA, USA). Norepinephrine, APV, propranolol, and glutamate were dissolved into a specific brain buffer which in 100 ml of volume 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 in 10% propylene glycol. The solvent dissolving a drug also served as the vehicle for control infusion.

To infuse a drug or vehicle solution, a piece of PE-20 polyethylene tubing was connected to a 10 μl Hamilton syringe on one end and cemented to a 30 Gauge dental needle bent at a length of 16 mm on the other end. The intake of drug solution from the needle was preceded by filling the tubing with first distilled water followed by a small air bubble which separated the drug and distilled water. Bilateral infusion was administered into the BNST of a conscious rat in a way to minimize the stress that a rat experienced. A rat was gently held and stylets were removed from the cannulae, then the infusion needles were inserted such that the tips protruded 1 mm beyond the cannulae. The solution was dispensed at a rate of 0.5 μl per min by a syringe pump (CMA/100, Carnegie Medicine, Stockholm, Sweden); the infused volume was 0.5 μl each side. At the end of infusion, the needle was left in the cannula for an additional min to allow diffusion of the liquid. After the needles were withdrawn, the stylets were immediately replaced to prevent back flow. The drug infusion was on average completed in 2.5 min. The extent of drug diffusion in this infusion protocol was estimated in a few rats by intra-BNST infusion of 0.5 μl Evans blue (0.25%).

In all experiments, agonists were applied shortly after training (within 1 min) to test the involvement of a neurotransmitter system. When an antagonist was used to evaluate the receptor specificity of an agonist action, it was dissolved with the agonist into the same solution; the cocktail was applied shortly after training. When drugs were used to assess the interaction between two transmitter systems, the antagonist for one type of receptor was first applied 5 min before training to assure better blockade of that system during training and then the agonist for another type of receptor was applied shortly after training to activate the other system.

2.4. Inhibitory avoidance task

The inhibitory avoidance apparatus was a trough-shape alley divided by a sliding door into two compartments, one was lit by a 20-W light bulb and the other was dark. After recovery from the brain surgery, all rats were first subjected to an acclimation procedure. It was placed into the lit side facing away from the sliding door. As the rat turned around, the sliding door was opened to allow free access to the dark side for 20 s before being retrieved. This was repeated for two more times before the rat returning to its home cage. In the training session on the next day, the rat was placed into the lit side. When the rat stepped into the dark side, it received an inescapable foot shock applied through a constant current shocker controlled by a timer (Lafayette Instruments, Model 80240 and Model 58010, Indiana, USA). The shock intensity and duration depicted in the individual experiments were selected such that they were optimal for demonstrating a retention enhancing or impairing effect. Shortly after the shock administration, the rat was removed from the alley to receive post-training drug infusion and then returned to its home cage. In the 1-day retention test, the rat was again placed into the lit side and the latency (in s) of stepping into the dark side was recorded as the retention score. If a rat did not step across in 600 s, the test trial was ended and a ceiling score of 600 was assigned.

2.5. Shock startle task

In order to evaluate the effect of drug on shock sensitivity, after the inhibitory avoidance test a group of rats were subjected to a shock startle task (Chen, Ho, & Liang, 2000). The rat was placed into a startle apparatus (San Diego Instrument, San Diego, USA) and constrained in a Plexiglas cylindrical tube (length 20 cm, diameter 10 cm) enclosed in a ventilated, sound-attenuating cabinet (38 × 38 × 55 cm). Two types of stimuli were presented: The acoustic stimuli were high-intensity white noise bursts delivered by a speaker 30 cm above the animal; the shock stimuli were square-wave direct current generated from a programmable shocker (TI 30, Coulburn Instrument, San Diego, USA), which were

scrambled and delivered to a grid floor consisting of eight metal rods inserted inside the cylinder. The startle response was measured for a period of 200 ms after initiation of the stimulus by a vibration sensor attached to the base of the Plexiglas tube. The vibration force was transduced into voltage, then digitized and recorded by a computer for further analyses. The startle amplitude of each trial is defined as the maximal vibration during the 200-ms period of measurement.

Upon receiving the drug infusion a rat was placed into the startle apparatus with a continuous 55 dB background noise. After a 5-min acclimation period, 45 startle trials were presented with an inter-trial interval of 30 s. Two series of stimuli were dispensed to elicit startle: one contained nine different intensities of 0.1-s electric shocks; the other contained six acoustic stimuli. Each session contained three blocks of trials. Each block was composed of an acoustic series (two trials each for 95, 105, and 115 dB white noise bursts with 40-ms duration) followed by a shock series (ranging from 0 to 1.6 mA with an incremental step of 0.2 mA). Different intensities for each stimulus modality were presented in a quasi-random order within a series. The total time of a test session elapsed from the start of acclimation was 28 min. If a treatment affects shock sensitivity, it should alter shock startle without compromising acoustic startle; conversely, if a treatment affects the motor reactivity to any kind of stimulus, it would alter both shock and acoustic startle.

2.6. Open field task

To assess the possible effect of some drugs on locomotor activity, a group of rats were subjected to an open field task after being tested in the inhibitory avoidance task. The rat was placed into a square arena ($76 \times 76 \times 37$ cm) 5 min after intra-BNST infusion of the drug, then its locomotor activity was monitored by a video camera for 15 min. The arena was divided into nine (3×3) square units and each unit had an area of 25×25 cm^2; the center unit was defined as the central zone and all other units were defined as the peripheral zone. Distance of the traveling path in the central and peripheral zones of the arena was measured and recorded by the

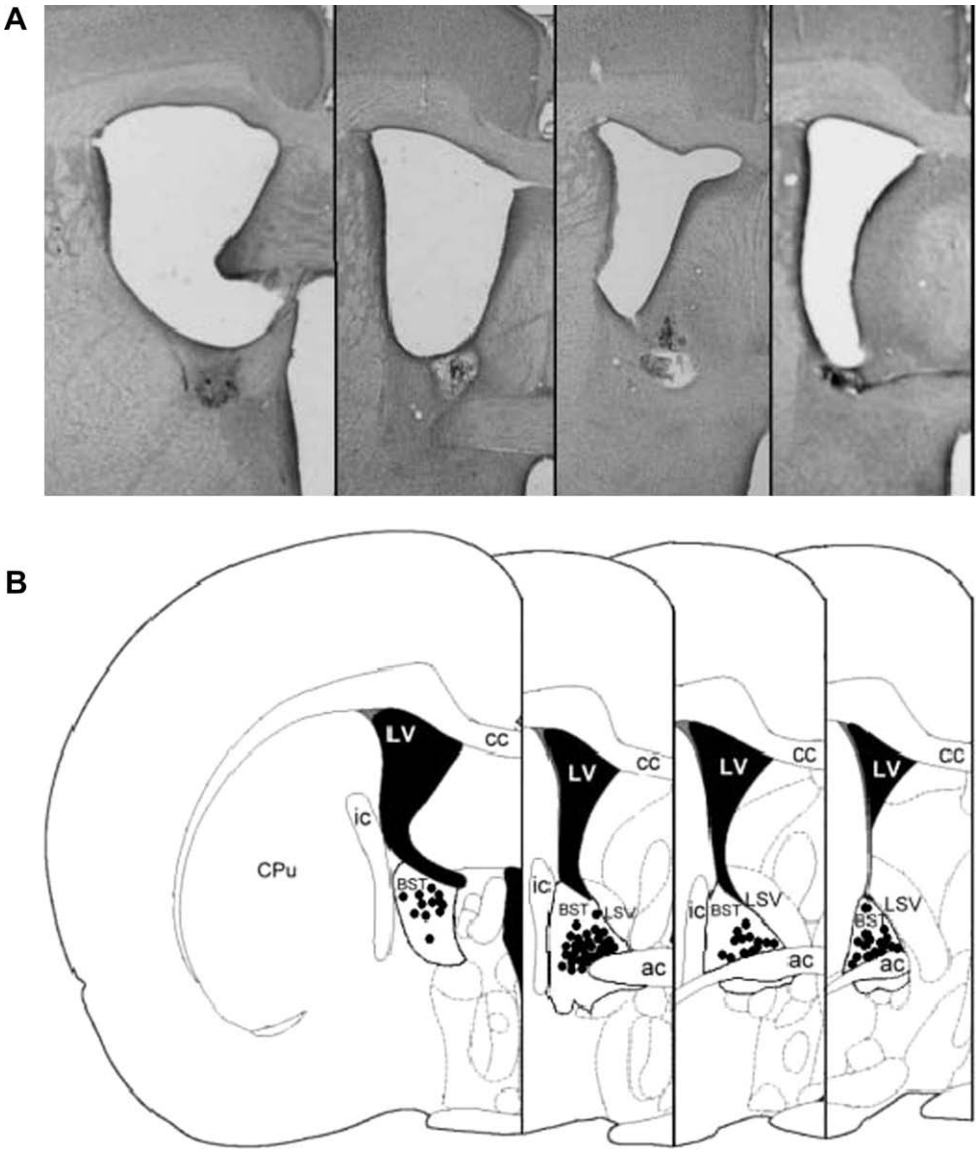

Fig. 1. (A) The photomicrograph of typical BNST cannula tips in four representative rats. (B) The distribution of cannula tips in a sample of experimental animals. The coronal brain plates are adapted with permission from the atlas of Paxinos and Watson (2005) (ac: anterior commissure; BST: bed nucleus of stria terminalis; cc: corpus callosum; CPu: caudate putamen; LSV: lateral septal nucleus, ventral part; LV: lateral ventricle; ic: internal capsule).

Ethovision system (Noldus Information Technology, Netherland) for each rat.

2.7. Histology

At the end of each experiment, rats were sacrificed with an overdose of sodium pentobarbital and perfused through the heart with physiological saline followed by 10% formalin. Then the brain was removed and stored in formalin with 20–25% sucrose for at least 48 h. The brain was sectioned (40 μm) and the brain slices were stained with cresyl violet. Placements of the cannulae were examined by projecting the stained slides onto coronal plates in the brain atlas of Paxinos and Watson (2005) and checking the location of the cannula tips on the plates. Animals were included in the data analysis if both needle placements were located within the BNST. A total of 429 rats were used in this study but 41 of them were excluded due to misplacement of cannulae. Fig. 1 shows the photomicrograph of cannula tips in four representative animals and the distribution of these tips in a sample of rats.

2.8. Data analysis

Because distribution of the retention score in an inhibitory avoidance task was truncated at 600 s, medians and inter-quartile ranges were used to represent the central and dispersion tendencies, respectively. The data were analyzed with nonparametric statistics: a Kruskal–Wallis one-way analysis of variance was used to detect an overall difference among groups followed by Mann–Whiney two-tailed *U*-tests to compare differences between group pairs.

For the shock sensitivity and locomotor activity data, the mean startle amplitude for various intensities in the two stimulus modalities and the movement distance in the 15-min period were calculated for each subject. These data were presented with the mean and standard error (SEM) and analyzed by parametric statistics. In the shock sensitivity test, two separate repeated-measure design two-way ANOVAs were conducted, respectively, for the shock and acoustic startle data with "Intensity" and "Drug" as the within-subject variables. In the locomotor activity test, the averaged moving distance per unit area (a 25×25-cm^2 square) in the central or peripheral zone of the open field was calculated for five successive blocks of 3 min in the 15-min period of observation. The data were analyzed by a three-way repeated-measure design ANOVA with "Drug", "Zone" and "Block" as the within-subject variables.

3. Results

3.1. Experiment 1: Post-training intra-BNST infusion of APV impaired retention

The first experiment examined the effect of intra-BNST infusion of APV on retention. Five groups of rats were trained with a 1.0 mA/1.0 s foot shock. Immediately after training, four groups received bilateral intra-BNST infusion of vehicle or APV at a dose of 0.2, 1.0 or 5.0 μg. The final group received infusion of 5.0 μg APV 4 h after training. Fig. 2 shows the results that post-training intra-BNST infusion of APV caused a dose- and time-dependent retention deficit. A significant difference was found among the groups ($H(4) = 10$, $p < .05$). Paired comparisons revealed that the 5.0 μg group had significantly poorer retention scores than the vehicle group ($U = 39$, $p < .05$), but no significant difference was detected between the vehicle group and the 0.2 or 1.0 μg group ($U = 58.5$ and 60 for the 0.2 and 1.0 μg groups, respectively; $p > .10$). The difference in retention scores between groups given immediate and delayed 5.0 μg APV infusion was statistically significant ($U = 33$, $p < .05$), but that between the vehicle and delayed APV infusion groups was not ($U = 115$, $p > .10$).

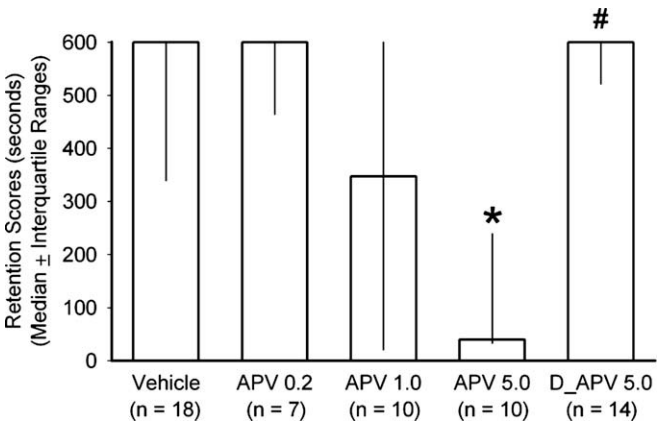

Fig. 2. Post-training intra-BNST infusion of 5.0 μg APV impaired retention (D denotes the 4 h-delay infusion, number of subjects in each group is noted in the parentheses). $^{*}p < .05$ different from the vehicle group; $^{\#}p < .05$ different from APV 5.0 μg group.

3.2. Experiment 2: Post-training intra-BNST infusion of glutamate enhanced retention

The second experiment examined the effect of intra-BNST infusion of glutamate on retention. Six groups of rats were trained with a 0.6 mA/0.6 s foot shock. Immediately after training, five of them received intra-BNST infusion of vehicle, glutamate at a dose of 0.2, 0.5 or 1.0 μg, or a cocktail of 1.0 μg glutamate plus 1.0 μg APV. The last group received infusion of 1.0 μg glutamate 4 h after training. Fig. 3 shows the results that intra-BNST infusion of glutamate caused dose- and time-dependent enhancement of retention, which was readily attenuated by APV concurrently infused into the same region. A significant difference was found among the groups ($H(5) = 16.21$, $p < .01$). The retention was significantly better for the group receiving 1.0 μg glutamate immediately after training than for the vehicle group ($U = 26$, $p < .01$). In contrast, retention in the group receiving 1.0 μg glutamate 4 h after training did not differ from that in the vehicle group ($U = 56$, $p > .10$) and was signifi-

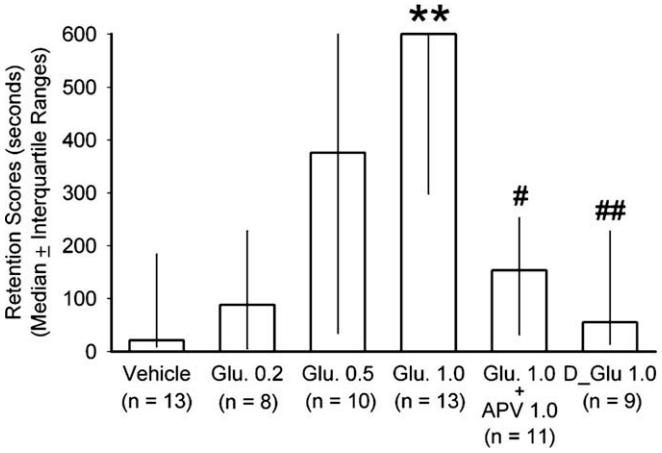

Fig. 3. Post-training intra-BNST infusion of 1.0 μg glutamate (Glu.) caused memory enhancement that was attenuated by 1.0 μg APV (D denotes the 4-h delay infusion, number of subjects in each group is noted in the parentheses). $^{*}p < .05$ different from the vehicle group; $^{\#}p < .05$ and $^{\#\#}p < .01$ different from the glutamate 1.0 μg group.

cantly poorer than that in the group receiving 1.0 μg glutamate immediately after training ($U = 12$, $p < .01$). Moreover, rats receiving 1.0 μg glutamate plus 1.0 μg APV had retention scores not different from those receiving vehicle ($U = 45$, $p > .10$), but significantly poorer than those receiving 1.0 μg glutamate ($U = 28$, $p < .05$).

3.3. Experiment 3: Post-training co-infusion of norepinephrine and glutamate into the BNST produced an additive memory enhancing effect

To explore whether glutamate interacted with norepinephrine to affect memory, four groups of rats were trained with a 0.6 mA/0.6 s foot shock and received one of the following treatments immediately after training: intra-BNST infusion of vehicle, 0.2 μg glutamate, 0.02 μg norepinephrine, or 0.02 μg norepinephrine plus 0.2 μg glutamate in a cocktail. Each drug was given at a sub-enhancing dose (Experiment 2 of this study; Liang et al., 2001) to facilitate the detection of any possible summative interaction between the two. Fig. 4 shows the results that norepinephrine or glutamate at the selected dose had no effect of its own, yet they produced marked memory enhancement if infused together; the difference among the groups was significant ($H(3) = 12.22$, $p < .01$). Paired comparisons indicated that the norepinephrine or glutamate group did not significantly differ in retention than the vehicle group ($U = 51$ and 87 for the norepinephrine and glutamate comparisons, respectively; $p > .10$). In contrast, the norepinephrine plus glutamate group showed significantly better retention than any other group ($U = 33$, 25 or 45 in comparison with the vehicle, norepinephrine or glutamate group, respectively; $p < .01$).

3.4. Experiment 4: Intra-BNST infusion of norepinephrine attenuated the amnesic effect of APV infused into the same region

This experiment tested if norepinephrine at a non-enhancing dose (Experiment 3 of this study) ameliorated the memory deficit induced by NMDA blockade. Three groups of rats trained under a 1.0 mA/1.0 s foot shock received one of the following three pre-training/post-training intra-BNST infusion treatments: vehicle/vehicle, 5.0 μg APV/vehicle, or 5.0 μg APV/0.02 μg norepinephrine. Fig. 5 shows the results that pre-training intra-BNST infusion of APV produced a significant memory deficit, which was attenuated by post-training infusion of norepinephrine. A significant difference was detected among the groups ($H(2) = 10.22$, $p < .01$). Paired comparisons showed that the vehicle/vehicle group had better retention than the APV/vehicle group ($U = 21$, $p < .01$). Retention in the APV/norepinephrine group were significantly better than that in the APV/vehicle group ($U = 36$, $p < .05$), but not different from that in the vehicle/vehicle group ($U = 57$, $p > .10$).

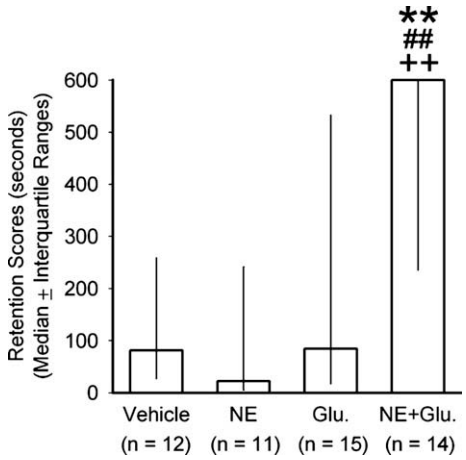

Fig. 4. Post-training co-infusion of 0.02 μg norepinephrine (NE) and 0.2 μg glutamate (Glu.) into the BNST induced a summative enhancing effect on retention. (Number of subjects in each group is noted in the parentheses.) $^{**}p < .01$, $^{\#\#}p < .01$, and $^{++}p < .01$ different from the vehicle, norepinephrine, and glutamate group, respectively.

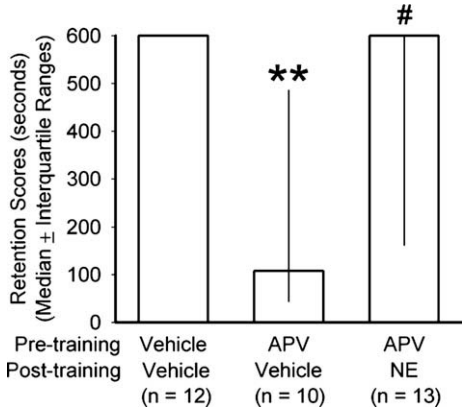

Fig. 5. Post-training intra-BNST infusion of 0.02 μg norepinephrine (NE) attenuated the retention deficit induced by pre-training infusion of 5.0 μg APV into the same region. (Number of subjects in each group is noted in the parentheses.) $^{**}p < .01$ different from the vehicle/vehicle group; $^{\#}p < .05$ different from the APV/Vehicle group.

3.5. Experiment 5: Post-training intra-BNST infusion of propranolol impaired retention and attenuated the memory enhancing effect of norepinephrine

This experiment investigated the involvement of the BNST β adrenoceptors in memory formation. It first examined the effect of post-training intra-BNST infusion of propranolol, a β adrenoceptor blocker, on retention. Five groups of rats were trained with a 1.0 mA/1.0 s foot shock. Immediately after training, four of them received bilateral intra-BNST infusion of vehicle or propranolol at a dose of 1.0, 5.0 or 10.0 μg. A final group received infusion of 10.0 μg propranolol 4 h after training. This was followed by examining whether propranolol attenuated the memory enhancing effect of norepinephrine. Three groups of rats were trained with a 0.6 mA/0.6 s foot shock. Immediately after training, they received bilateral intra-BNST infusion of vehicle, 1.0 μg norepinephrine, or 1.0 μg norepinephrine plus 5.0 μg propranolol in a cocktail.

Fig. 6A shows the first part of the results: post-training intra-BNST infusion of propranolol caused a dose- and time-dependent retention deficit, the difference among various groups was significant ($H(4) = 9.52$, $p < .05$). Paired comparisons indicated that the 10.0 μg propranolol group had retention poorer than the vehicle group ($U = 26.5$, $p < .05$), while the 1.0 or 5.0 μg propranolol group did not differ from the vehicle group ($U = 37.5$ and 36.5 for the 1.0 and 5.0 μg propranolol groups, respectively; $p > .10$). The difference between groups receiving immediate and delayed infusion of 10.0 μg propranolol was significant ($U = 43$, $p < .05$), but that between the vehicle and delayed infusion groups was not ($U = 58$, $p > .10$).

Fig. 6B shows the second part of results: post-training intra-BNST infusion of 1.0 μg norepinephrine enhanced retention that was attenuated by 5.0 μg propranolol simultaneously infused into the same region; the differences among various groups was significant ($H(2) = 9.42$, $p < .01$). Paired comparisons indicated that rats receiving norepinephrine had retention better than those receiving vehicle ($U = 11$, $p < .01$). Rats receiving norepinephrine plus propranolol did not differ from the vehicle group ($U = 30$, $p > .10$),

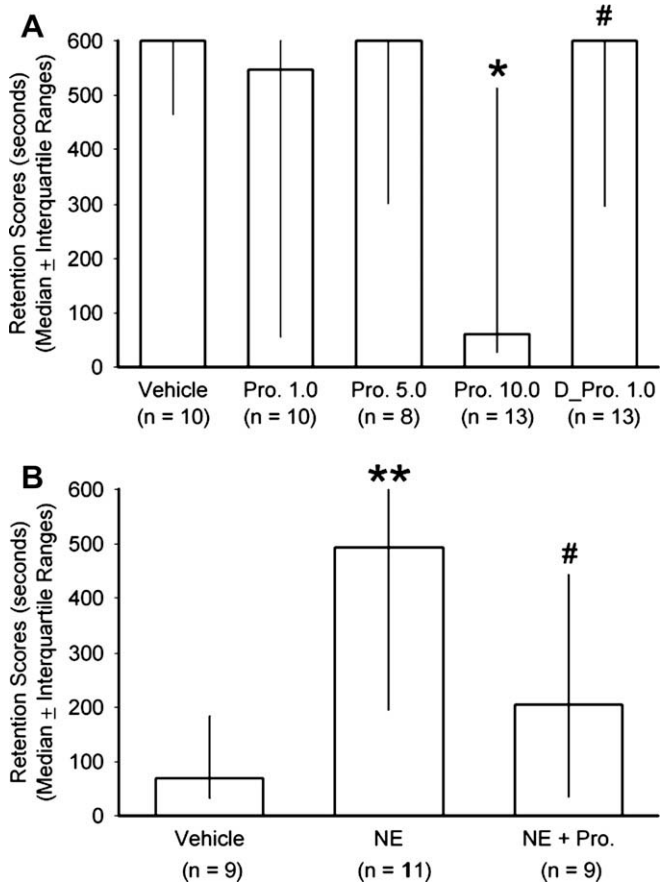

Fig. 6. (A) Post-training intra-BNST infusion of 10.0 μg propranolol (Pro.) impaired the retention. $^{*}p < .05$ different from the vehicle group; $^{\#}p < .05$ different from the propranolol 10.0 μg group. (B) Post-training intra-BNST infusion of 1.0 μg norepinephrine (NE) induced retention enhancement that was attenuated by simultaneous infusion of 5.0 μg propranolol (Pro.). $^{**}p < .01$ different from the vehicle group. $^{\#}p < .05$ different from the NE group (D denotes the 4 h-delay infusion, number of subjects in each group is noted in the parentheses).

but had poorer retention scores than those receiving norepinephrine ($U = 23$, $p < .05$).

3.6. Experiment 6: Intra-BNST infusion of propranolol but not prazosin attenuated the memory enhancing effect of glutamate

This experiment tested the involvement of α_1 or β adrenoceptors in the memory enhancing effect of glutamate. Five groups of rats were trained with a 0.6 mA/0.6 s foot shock and received one of the following pre-training/post-training intra-BNST infusion treatments: vehicle/vehicle, vehicle/1.0 μg glutamate, 0.3 μg prazosin/1.0 μg glutamate, 3.0 μg prazosin/1.0 μg glutamate, and 5.0 μg propranolol/1.0 μg glutamate. The doses of prazosin and propranolol were selected for not causing apparent amnesia of their own based on the results of Experiment 5 and a previous study (Liang et al., 2001) to test if these two drugs at a non-impairing dose could attenuate the memory enhancement induced by glutamate.

Fig. 7 shows the results that intra-BNST infusion of glutamate produced marked memory enhancement that was attenuated by propranolol but not by prazosin. The difference among the groups was significant ($H(4) = 39.56$, $p < .0001$). Paired comparisons showed that the vehicle/glutamate group had significantly better retention than the vehicle/vehicle group ($U = 59$, $p < .0001$). The 0.3 or 3.0 μg prazosin/glutamate group also had significantly better retention than the vehicle/vehicle group ($U = 109$, $p < .01$ for the

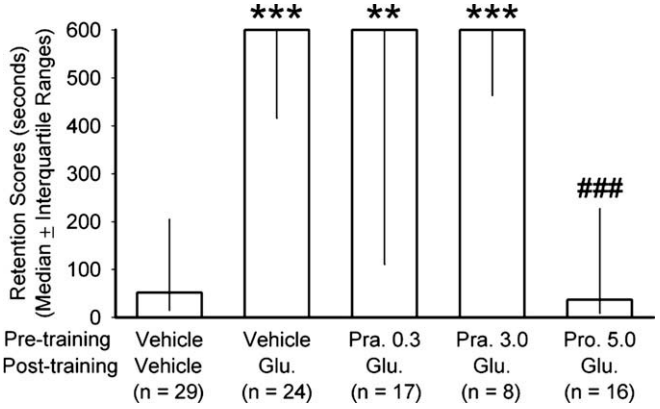

Fig. 7. Post-training intra-BNST infusion of 1.0 μg glutamate (Glu.) produced a marked memory enhancing effect, which was attenuated by pre-training infusion of 5.0 μg propranolol (Pro.) but not by 0.3 or 3.0 μg prazosin (Pra.). (Number of subjects in each group is noted in the parentheses.) $^{**}p < .01$ and $^{***}p < .001$ different from the vehicle/vehicle group. $^{\#\#\#}p < .001$ different from the vehicle/glutamate group.

0.3 μg prazosin/glutamate group; $U = 19$, $p < .0001$ for the 3.0 μg prazosin/glutamate group), but neither of them differed from the vehicle/glutamate group ($U = 184$ and 95, respectively, for the 0.3 and 3.0 μg prazosin/glutamate groups, $p > .10$). Conversely, the 5.0 μg propranolol/glutamate group had poorer retention than the vehicle/glutamate group ($U = 42$, $p < .0001$), but did not significantly differ from the vehicle/vehicle group ($U = 227.5$, $p > .10$).

3.7. Experiment 7: Intra-BNST infusion of APV or propranolol did not influence shock sensitivity and locomotor activity

The final experiment assessed the possible influence of those drugs given before a training trial on shock sensitivity and locomotor activity. In the first part of this experiment, rats received bilateral infusion of vehicle, 5.0 μg APV, and 10.0 μg propranolol into the BNST for three consecutive days in a counter-balanced sequence. Immediately after drug infusion, their startle responses to shock and acoustic stimuli were tested. The results are shown in Fig. 8A: pretesting intra-BNST infusion of APV or propranolol did not influence shock and acoustic startle. Repeated-measure design two-way ANOVAs showed that the "Intensity" main effect was significant for either shock startle ($F(8,88) = 39.93$, $p < .001$) or acoustic startle ($F(2,22) = 6.94$, $p < .01$), suggesting that the startle amplitude increased with shock or sound intensities. On the other hand, the "Drug" main effect and the "Drug × Intensity" interaction effect were not significant for shock startle ($F(2,22) < 1$ and $F(16,176) < 1$ for the main effect and interaction effects, respectively) and acoustic startle ($F(2,22) = 1.61$, $p > .10$ for the main effect; $F(4,44) < 1$ for the interaction effect).

The second part of this experiment evaluated drug effects on locomotor activity in an open field task. Another group of rats received bilateral infusion of vehicle, 5.0 μg APV, and 10.0 μg propranolol into the BNST for three consecutive days in a counter-balanced sequence. After the drug infusion, rats were placed into the arena and their locomotor activities were measured for 15 min. The mean moving distance per unit area at the central or peripheral zone for successive time blocks in the 15-min observation period is shown in Fig. 8B: pretesting intra-BNST infusion of APV or propranolol did not affect locomotor activity at the central and peripheral zones. A three-way ANOVA with a repeated-measure design revealed a significant "Block" main effect ($F(4,44) = 6.48$, $p < .0001$), indicative of a decreasing trend in overall locomotion along with time. The "Drug" or "Zone" main effect was not significant ($F(2,22) < 1$ and $F(1,11) < 1$, respectively). No

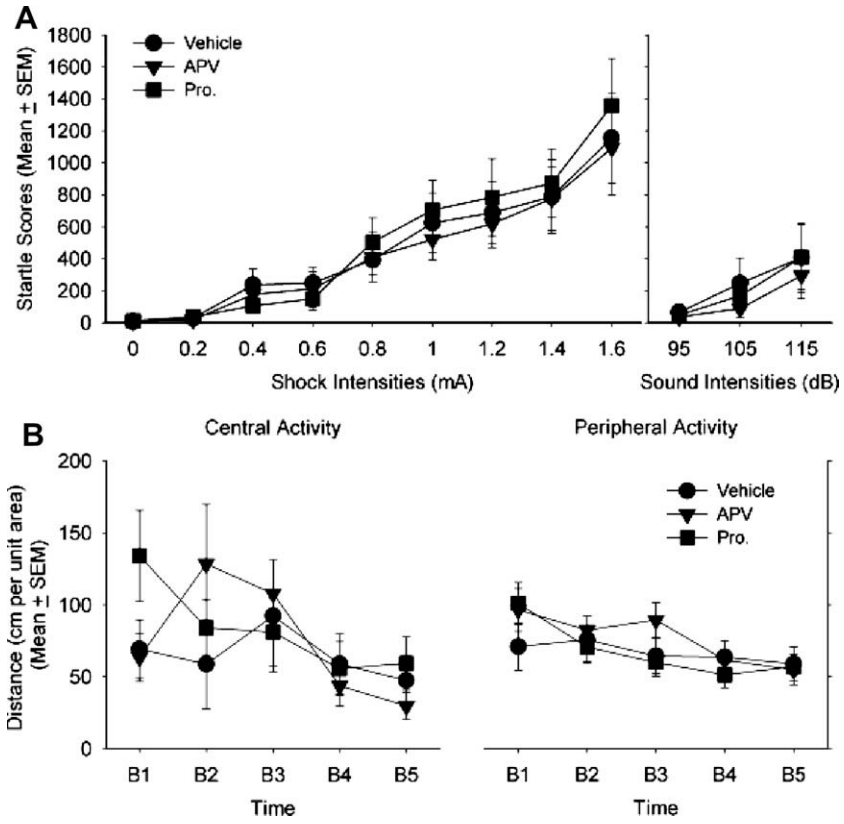

Fig. 8. (A) Intra-BNST infusion of 5.0 μg APV and 10.0 μg propranolol (Pro.) did not affect the startle response to shocks (left panel) or acoustic stimuli (right panel) ($n = 12$). (B) Intra-BNST infusion of 5.0 μg APV and 10.0 μg propranolol (Pro.) did not affect the averaged locomotor activity in an unit area (a square of 25 × 25 cm^2) at the central zone (left panel) or peripheral zone (right panel) of an open field for the 15-min observation period ($n = 12$) (B1–B5 denote five consecutive blocks of 3 min).

interaction effects reached statistical significance ($F(8,88) = 1.76$, $p > .05$ for the "Drug × Block" effect; $F(4,44) = 2.29$, $p > .05$ for the "Block × Zone" effect; $F(2,22) < 1$, for the Drug × Zone effect; $F(8,88) = 1.53$, $p > .05$ for the "Drug × Block × Zone" effect).

4. Discussion

Several major findings emerged from this study: first, in an inhibitory avoidance task post-training intra-BNST infusion of APV or glutamate, respectively, impaired or enhanced retention; and APV blocked the enhancing effect of glutamate. Second, concurrent infusion of norepinephrine and glutamate into the BNST after training produced an additive memory enhancing effect, and post-training infusion of norepinephrine ameliorated the amnesic effect of APV. Third, intra-BNST infusion of a β-adrenergic antagonist propranolol not only impaired retention, but attenuated the memory enhancing effects of both norepinephrine and glutamate. These findings suggest that glutamate interacted with norepinephrine in the BNST to affect memory processing of an inhibitory avoidance response.

This study adopted a post-training infusion regimen in most experiments and obtained robust time-dependent effects for glutamate, APV and propranolol. It thus convincingly ruled out a contribution of performance factors to the observed effects. For the experiments in which antagonists were administered before training, the drug might have affected motivational or sensory-motor factors during acquisition. The present and previous results (Liang et al., 2001) showed that the antagonists infused into the BNST did not affect shock sensitivity in a startle test or locomotor activity at the central or peripheral zones of an open field. The latter results also ruled out the possibility that the drug might cause the effect by regulating anxiety states, a function often ascribed to the BNST. Finally, the attenuating agent given in this study was at a dose lacking any effect of its own on memory; thus it is unlikely that the attenuation effect is due to summation of two actions opposite in sign but irrelevant in mechanism. These data altogether suggest that the observed effect should be mainly due to altered memory formation processing (McGaugh, 2000). This suggestion is consistent with our previous results that lidocaine infused into the BNST after training impaired consolidation of memory in the adopted task (Liang et al., 2001).

The present study showed that post-training intra-BNST infusion of glutamate induced dose- and time-dependent memory enhancement in the inhibitory avoidance task, consistent with the effects on memory of glutamate infused into several other brain regions (Clayton & Williams, 2000; De Leonibus et al., 2003; LaLumiere, Pizano, & McGaugh, 2004; Liang, Hon, & Davis, 1994; Roesler et al., 2003). The attenuation of the glutamate-induced memory enhancement by a non-impairing dose of APV attests to the involvement of NMDA receptors in the glutamate effect. The amnesia induced by APV at a higher dose suggests that under natural conditions inhibitory avoidance training may release endogenous glutamate into the BNST acting on NMDA receptors to affect memory formation.

The present findings support a vital role of norepinephrine affluent in the BNST in modulating aversive memory. In previous studies, we have shown that the BNST norepinephrine facilitated acquisition and/or retention in the inhibitory avoidance task and Morris water maze by acting through α_1, but not α_2 adrenoceptors (Chen et al., 2004; Liang et al., 2001). The present data showed that a high dose (10.0 μg) of propranolol induced a pronounced retention deficit but a lower and non-impairing dose (5.0 μg) of it

attenuated the memory enhancing effect of norepinephrine. These findings extend the previous ones by suggesting that memory enhancement induced by norepinephrine in the BNST involves both α_1 and β adrenoceptors. Similar findings have also been reported for the amygdala in which the influence on memory formation of α_1 receptors depends upon the β receptor activation (Ferry, Roozendaal, & McGaugh, 1999a, 1999b), it would be of interests to pursue whether this would also be the case in the BNST.

Three lines of evidence yielded in this study support, but not necessarily prove, an interaction between glutamate and norepinephrine in the BNST to modulate memory formation. First, co-infusion of glutamate and norepinephrine into the BNST caused an additive memory enhancing effect. Second, the amnesic effect of APV infused into the BNST was ameliorated by norepinephrine infused into the same region. Third, propranolol attenuated the memory enhancement induced by infusion of norepinephrine or glutamate into the BNST. Previous studies have shown that activation of NMDA receptors induced release of norepinephrine in brain regions such as the BNST, hippocampus, and cerebral cortex (Andres, Bustos, & Gysling, 1993; Fink, Bonisch, & Gothert, 1990; Forray et al., 1995; Pittaluga & Raiteri, 1990). Moreover, noradrenergic neurons projecting to the BNST expressed mRNA for the NMDA-NR1 receptors (Forray et al., 2000); such receptors are present at the axon terminals (Gracy & Pickel, 1995) and thus capable of mediating the effect of locally infused glutamate in the BNST.

Thus, these and previous findings provide convergent evidence consistent with an interpretation that glutamate enhanced retention by releasing norepinephrine in the BNST, although a possibility remains viable that the two neurotransmitters might act independently in the BNST to modulate memory. Noradrenergic fibers innervate the ventral BNST more densely (Phelix et al., 1992) but both α_1 and β adrenoceptors are present in the dorsal BNST as well (Egli et al., 2005; McElligott & Winder, 2008). Given a diffusion range of 1.0 mm for 0.5 μl infusion volume estimated from dye spread in this and a former studies (Liu & Liang, in press), drugs infused into the locations of this study would encroach into most BNST nuclei and affect the region rich in noradrenergic terminals or receptors. Mediation of the glutamate effect on memory by norepinephrine has been proposed to act in the amygdala (Ferry, Magistretti, & Pralong, 1997; Lennartz, Hellems, Mook, & Gold, 1996; Liang, Lin, & Tyan, 1993). In view of the biphasic influences of norepinephrine on memory (Dalmaz, Introini-Collison, & McGaugh, 1993; Liang et al., 1990), this mode of glutamate action on memory can readily account for findings that APV infused into the brain could enhance or impair retention depending on various factors (LaLumiere et al., 2004).

An intriguing discrepancy between this and the previous studies is that the memory enhancing effect of exogenously infused norepinephrine involves both α_1 and β adrenoceptors (Chen et al., 2004; Liang et al., 2001; this study, Experiment 5), yet the effect of endogenous norepinephrine released by glutamate stimulation on NMDA receptors involves only β but not α_1 adrenoceptors (this study, Experiment 6). In the dorsal hippocampus, endogenous norepinephrine released by stimulation of the locus coeruleus and exogenous norepinephrine given by microiontophoresis caused distinct neurophysiological responses that were attributed to activating adrenoceptors inside or outside the synaptic zone (Curet & de Montigny, 1988a, 1988b), and differential density distribution of α_1 and β adrenoceptor was indeed found in the hippocampus (Duncan et al., 1991; Zilles et al., 1991). Differential involvement of the BNST α_1 and β receptors in the effects observed by our studies might be potentially due to, among others, that the exogenously infused norepinephrine acts on synaptic as well as extrasynaptic receptors but the endogenously released norepinephrine acts mainly on the synaptic ones. To uphold this account, α_1 and β receptors must have differential extra-synaptic and synaptic distributions within the BNST —a postulate awaiting further evidence for support.

The present findings are consistent with a role of the BNST in aversive or appetitive learning as shown by other studies (Dumont et al., 2005; Gray et al., 1993; Schweimer et al., 2005; Sullivan et al., 2004). Evidence has shown that the BNST may or may not be involved in retrieval of conditioned fear memory depending on the nature of the task or other factors (Davis, Walker, & Lee, 1997; Schweimer et al., 2005; Sullivan et al., 2004). Our previous data showed that the BNST was not involved in memory expression for the inhibitory avoidance task and the Morris water maze (Chen et al., 2004; Liang et al., 2001). Abundance of evidence has shown that the BNST is activated by unconditioned fear stimuli (Davis et al., 1997; Fendt, Endres, & Apfelbach, 2003), stressors of various sorts (Spencer & Day, 2004) or anxiogenic drugs (Singewald, Salchner, & Sharp, 2003). Sensitivity of the BNST to stress may allow the nuclei to be activated by an aversive event and hence to modulate memory formation for that event or any other learning accompanied with it. An example for the latter case is the recent findings that the BNST is critical for stress modulation of Pavlovian eyelid conditioning in male rats (Bangasser & Shors, 2008).

The input–output circuitry underlying the BNST memory function can only be speculated. The BNST receives convergent input from the hippocampal formation and amygdala (Canteras & Swanson, 1992; Dong, Petrovich, & Swanson, 2001), both of which are implicated in processing inhibitory avoidance memory (Chang, Liang, & Yen, 2005; Izquierdo et al., 1992; Liang, Tsui, Tyan, & Chiang, 1998; Liang et al., 1994). Glutamatergic fibers project to the BNST directly from the ventral subiculum via the precommissural fornix (Walaas & Fonnum, 1980) and may interact with the BNST noradrenergic fibers to affect memory formation (Liu & Liang, in press). Alternatively, the ST fibers from the amygdala containing γ-amino-butyric acid or various neuropeptides (Le Gal LaSalle, Paxinos, & Ben-Ari, 1978; Roberts, Woodhams, Crow, & Polak, 1980; Sakanaka, Shibasaki, & Lederis, 1986; Sakanaka et al., 1981; Uhl & Snyder, 1979; Uhl et al., 1978) may instigate the action of norepinephrine and glutamate on the BNST observed in this study. Memory processing in the inhibitory avoidance task also involves the medial prefrontal or anterior cingulate cortex (Izquierdo et al., 2007; Liang, Hu, & Chang, 1996; Malin, Ibrahim, Tu, & McGaugh, 2007), nucleus accumbens (Roozendaal, de Quervain, Ferry, Setlow, & McGaugh, 2001) and other brain structures. Through its profuse reciprocal connections with the amygdala and nucleus accumbens (De Olmos, Beltramino, & Alheid, 2004), the BNST may be, along with other engaged structures, part of a reverberating circuit to modulate consolidation of the inhibitory avoidance memory. Whether such an action involves the neural plasticity detected in the BNST (Weitlauf, Egli, Grueter, & Winder, 2004) is another issue awaiting further research.

Acknowledgments

The present study was supported by the funding of NSC-94-2413-H-002-001-, NSC-94-2752-H-002-006-PAE, and other Grants from National Science Council, Taiwan, ROC to K. C. Liang. The authors would like to thank all the staff members and undergraduate students involved in assisting animal care and experimental work.

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