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The Bed Nucleus of the Stria Terminalis Is Critically Involved in Enhancing Associative Learning After Stressful Experience

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Abstract

Exposure to an acute stressful event enhances trace eyeblink conditioning in male rats, even when rats begin training days after the stressor (Shors, 2001). The authors examined whether the bed nucleus of the stria terminalis (BNST), an area involved in stress and anxiety, is critically involved in this effect and, if so, when. The authors found that excitotoxic lesions to the BNST prevented the enhanced conditioning after stressor exposure. In addition, temporary inactivation of the BNST during the stressor did not alter enhanced responding, whereas inactivation during training prevented the enhancement. These data indicate that stressful experience induces persistent changes in the BNST that are necessary for enhancing learning well after the stressful event has ceased.

Keywords

anxiety; amygdala; eyeblink; corticosterone; classical conditioning

Stressful events have been shown to influence the formation of new memories (for review, see Maier, 1984; McGaugh & Roozendaal, 2002; Shors, 2004). In male rats, exposure to an acute stressor of either periodic tailshocks or forced swimming greatly facilitates classical eyeblink conditioning (Beylin & Shors, 1998; Servatius & Shors, 1994; Shors, 2001; Shors & Servatius, 1995, 1997; Shors, Weiss, & Thompson, 1992). Exposure to these stressors also increases the release of corticosterone from the adrenal glands (Shors, 2001), and their removal prevents the facilitating effect of stress on eyeblink conditioning (Beylin & Shors, 2003). However, this effect still occurs when training begins 24 hr after stressor cessation (Servatius & Shors, 1994; Shors & Servatius, 1995), which is well after the stress-induced corticosterone response is over (Shors, Pickett, Wood, & Paczynski, 1999). In fact, the enhancement is expressed even when stressor exposure and training are separated by 4 days if the subjects are stressed and trained in the same context (Shors & Servatius, 1997). These findings suggest that, although the corticosterone response at the time of the stressor is necessary for this effect, the later expression of enhanced conditioning cannot be attributed to high corticosterone levels at the time of training. Thus, it appears that the stress-induced release of corticosterone initiates a long-lasting process that mediates the enhanced conditioning over days.

Little is known about what structures contribute to this effect of stress on learning. However, it has been shown that activation of *N*-methyl-D-aspartate (NMDA) receptors within the basolateral-lateral nuclei of the amygdala during the stressor, but not after, is necessary for

inducing the effect of stress on conditioning (Shors & Mathew, 1998). The amygdala projects heavily to the bed nucleus of the stria terminalis (BNST; Alheid, 2003; Krettek & Price, 1978; Weller & Smith, 1982), an area known to be involved in a number of anxiety and stresslike behaviors (Casada & Dafny, 1991; Davis & Shi, 1999; Davis, Walker, & Lee, 1997; Walker, Toufexis, & Davis, 2003). The current set of experiments examined the role of the BNST in the effect of stress on trace eyeblink conditioning. In Experiment 1, we determined whether the BNST was critically involved in the enhanced conditioning. In Experiment 2, we determined when it was involved: during the stressor or during training.

Method

Experiment 1

Subjects—Male Sprague–Dawley rats (60–90 days) were obtained from Zivic-Miller (Pittsburgh, PA) or bred from Zivic-Miller stock and housed alone in single wire cages. Rats had ad lib access to food and water and were maintained on a 12-hr light–dark cycle.

Surgery—Rats were anesthetized with a small dose of sodium pentobarbital (12 mg/kg) and maintained on isoflurane and oxygen. After being placed in a stereotaxic instrument, the scalp was cleaned with Betadine, an incision was made, and the skull above the target region was removed with a dental bur. The needle of a 1- μ L Hamilton syringe was lowered into the BNST (AP -0.4 mm relative to bregma, ML ± 1.4 mm from the midline, and DV -6.4 mm from the surface of the brain) as determined by the rat brain atlas (Paxinos & Watson, 1997). NMDA (15 mg/ μ L in phosphate-buffered saline, pH 7.4) was infused bilaterally at a volume of 0.08 μ L/side. The flow rate (0.02 μ L/min) was controlled by a microinfusion pump (Stoelting, Chicago, IL). After infusion, the needle was left in place for 5 min to allow for diffusion of the compound. For sham controls, a needle was lowered to 0.5 mm above the BNST (DV -5.9) for 10 min. Head stages were mounted onto the skull in preparation for eyeblink conditioning. Two pairs of electrodes (insulated stainless steel wire 0.005 in.) were attached to a head stage and implanted through the upper eyelid (orbicularis oculi muscle). One electrode from each pair was placed within the muscle itself, and the second electrode was placed just outside the muscle. The insulation was removed from all electrodes to ensure contact with the muscle or connective tissue. The head stage was attached to the skull with dental cement and four anchoring screws. Rats were allowed at least 1 week to recover before training.

Conditioning apparatus—The conditioning apparatus consisted of an inner chamber (22 cm $\times$ 26 cm $\times$ 25 cm) with metal walls and a grounded floor grid within a sound-attenuating outer chamber (51 cm $\times$ 52 cm $\times$ 35 cm). A program written in VIEWDAC controlled a white noise generator attached to a speaker that was used to administer an 83-dB white noise conditioned stimulus (CS). A shock generator (Lafayette Instruments, Lafayette, IN) was used to administer the 0.40-mA eyelid shock unconditioned stimulus (US). The chamber was illuminated with a 7.5-W bulb. Ten conditioning chambers were used simultaneously.

Recording and analysis of eyeblinks—Eyeblinks were detected by changes in eyelid electromyographic (EMG) activity. EMG electrodes were connected to a differential amplifier with a 300- to 500-Hz bandpass filter and amplified 10,000 times. EMG signals were digitized at 1 kHz and analyzed with a VIEWDAC software program. EMG activity for each trial was compared with a 250-ms pre-CS baseline. Eyeblinks were defined as changes in EMG activity that exceeded the maximum amplitude in this baseline by at least 4 *SD* for a minimum duration of 3 ms.

Acclimation and stressor exposure—After at least 1 week of recovery, rats were placed in the conditioning chamber for 40 min to acclimate them to the conditioning environment. No stimuli were presented during this time. After acclimation, some rats were returned to their home cages, and those selected to be in a stress group were taken into another room and put in a restraining tube located within a dark sound-attenuating chamber. Electrodes were attached to the tail to deliver 30 shocks (1 s, 1 mA) at a rate of 1/min.

Trace eyeblink conditioning—Twenty-four hours after acclimation (and stressor exposure for some), rats were returned to the conditioning chamber, and baseline blink rates were assessed by recording responses over thirty 500-ms dummy trials during which no stimuli were presented, for a total recording period of 15 s over 10 min, as described previously (Shors & Servatius, 1997). Rats were then exposed to ten 250-ms CS-alone presentations (with an intertrial interval of 25 ± 5 s), and blinks were recorded for a 500-ms period after each CS. Previous studies have shown that this procedure is sensitive enough to detect differences in reactivity to the CS before conditioning (Shors & Servatius, 1995). This was followed by training with a trace eyeblink conditioning task (200 trials/day for 2 days). In the trace conditioning paradigm, the CS (250 ms, 83 dB) was separated from the 100-ms, periorbital shock US (0.40 mA) by a 500-ms trace interval. The volume of the CS and intensity of the shock US were chosen to produce a slow rate of acquisition and thus enhance the possibility of observing an increase in conditioned responding as a result of stressor exposure. Trials were presented in blocks of 10 in the following order: one CS-alone trial, four paired trials, one US-alone trial, and four paired trials, with an intertrial interval of 25 ± 5 s. Conditioned responses (CRs) were eyeblinks that occurred during the 500-ms trace interval on paired trials and 750 ms after the CS offset on CS-alone trials. The percentage of CRs emitted was analyzed with a mixed-factor analysis of variance (ANOVA) and Newman–Keuls post hoc comparisons.

Histology—After training, rats were deeply anesthetized and transcardially perfused with 10% formalin. Brains were extracted and then post-fixed for approximately 6 days in a 2% ammonium bromide and 10% formalin solution with sucrose (final concentration 30%) on the last 3 days. Coronal sections (40 μ m) containing target regions were cut with a freezing microtome. Every other section was stained with Cajal's gold sublimate method for astrocytes, which detects reactive gliosis (Figure 1A). Lesions were indicated by the absence of nerve cell bodies and the presence of darkly stained astrocytes. A rater, unaware of experimental condition, made qualitative lesion assessments. Rats were excluded if they had unilateral lesions ($n = 2$), if the compound leaked into the ventricles as indicated by reactive gliosis around the ventricles ($n = 3$), or if the lesions were too posterior, damaging the reticular thalamic nucleus and the stria medullaris ($n = 2$). Rats included in the study had bilateral damage throughout the BNST. The extent of the smallest lesion and largest lesion included in the study is depicted in Figure 1B. These criteria resulted in 6 unstressed sham rats, 7 stressed sham rats, 7 unstressed lesioned rats, and 7 stressed lesioned rats, for a total sample of 27 in this experiment.

Experiment 2

Surgery—Anesthesia for the surgery was the same as in Experiment 1. Guide cannulas (23 gauge, Plastics One, Roanoke, VA) were implanted bilaterally, along with stylets (tips flush with guides) aimed at the BNST (AP -0.4 mm relative to bregma; ML ± 1.4 mm from the midline; and the depth was -6.4 mm from the surface of the brain, angled 15° toward midline to avoid puncturing the ventricles). Cannulas and head stages were fixed to the skull with dental cement and four anchoring screws, and eyelid electrodes were implanted as in Experiment 1.

Transient inactivation of the BNST—Rats were handled 3 min/day for 3 days before surgery and 10 min/day for 3 days after surgery to minimize the stressful aspects of the microinfusion procedures. During the infusions, stylets were replaced with infusion cannulas extending 1 mm beyond the tip of the guide cannula. Infusion cannulas were connected to a microinfusion pump by means of polyethylene tubes attached to 10- μ L Hamilton syringes. The entire system was backloaded with distilled water, with a small air bubble separating the distilled water from the drug or vehicle solution. To temporarily inactivate the BNST, muscimol, a gamma aminobutyric acid A receptor agonist, was bilaterally infused (250 nl/side at a rate of 0.05 μ L/min; 1 ng/nl in artificial cerebrospinal fluid [aCSF], pH 7.4). For control infusions vehicle (aCSF) alone was infused. After infusion of the compound, cannulas were left in place for 2 min to allow for proper diffusion. Concentration and volume were estimated from previous studies in which muscimol was infused into the BNST (Fendt, Endres, & Apfelbach, 2003) and the central and basolateral nuclei of the amygdala (Manning, 1998; Sanders & Shekhar, 1995).

Acclimation, stressor exposure, and conditioning procedure—Because the peak efficacy of muscimol diminishes after 1 hr (Martin, 1991), rats were trained for 100 trials a day (session length of about 40 min) for 4 days. All other parameters and analyses were as in Experiment 1. Four experimental conditions were used as outlined in Figure 2A: the first group (inactivation during stressor exposure) received one muscimol infusion before stressor exposure and vehicle (aCSF) infusions before each day of training; the second group (stress–inactivation during training) received vehicle infusion before stressor exposure and muscimol infusion before each day of training; the third group (stress) received vehicle infusions before stressor exposure and before each day of training; the final group (no stress) received vehicle infusion the day before training (when stressor exposure would have occurred) and vehicle infusions before each day of training.

As noted earlier, training parameters that would yield low levels of conditioning in the unstressed rats were purposely selected. Because of this, it was important to verify that the BNST inactivation during training did not inhibit trace conditioning itself irrespective of stressor exposure. This issue was addressed in two ways. First, we examined the effects of muscimol infusion on performance in rats that had already learned the task. To do that, a subset of rats ($n = 6$) that had vehicle infused during training were exposed to 100 additional trials of trace conditioning while the BNST was inactivated. It was reasoned that if muscimol infusion disrupts performance of the conditioned eyeblink response, then the CRs would decrease in this group. This subset of rats was selected because they had a high rate of responding during training ($> 40\%$ CRs), and thus a decrease in responding after muscimol infusion could be observed. This subset of rats included 1 rat from the group that was not stressed, 2 from the group that was stressed, and 3 from the group that was stressed while the BNST was inactivated. None of these rats had been infused with muscimol during training before this manipulation.

Second, to verify that the BNST inactivation during training did not inhibit trace conditioning itself irrespective of stressor exposure, we addressed whether muscimol affected the acquisition of the CR. To do that, rats that had low levels of conditioned responding during training were chosen. The BNST was inactivated with muscimol, and they were then exposed to 100 additional trials of training using a more intense US (0.65 mA, $n = 5$). These parameters have previously been shown to enhance conditioned responding in unstressed males (Bangasser & Shors, 2005). The percentage of CRs in these rats was compared with that obtained from unstressed rats that continued to receive vehicle infusions during 100 additional trials of training with the more intense US ($n = 4$). The unstressed group was used as a comparison because they also had low levels of responding during training. If BNST inactivation impairs or prevents acquisition of eyeblink

conditioning itself, independent of the effect of stress, then training with a higher intensity US should not elicit more CRs. If, alternatively, BNST inactivation does impair performance, these rats should emit few CRs.

Histology and corticosterone assay—We had previously shown that the stress-induced release of glucocorticoids is necessary for the enhanced conditioning after stressor exposure (Beylin & Shors, 2003). Thus, we were concerned that BNST inactivation might alter the amount or degree of hypothalamic–pituitary–adrenal activation. To evaluate this issue, at least 5 days after the initial stressor, we exposed the stressed rats once again to the stressor and measured the release of corticosterone while the BNST was inactivated. Although it is possible that the corticosterone response would be different on reexposure to the second stressor, most studies find that the response is similar if elicited again days after the first event (O'Connor et al., 2004). Rats were anesthetized with sodium pentobarbital and then infused bilaterally with 4% methylene blue dye (250 nl), which marked the location of the cannula tips. Trunk blood was collected (approximately 20 min after stressor cessation), and brains were quickly extracted and post-fixed in 10% formalin. Blood was centrifuged and serum was frozen (−4 °C) for later radioimmunoassay (RIA) of corticosterone. Three days before cutting, brains were transferred to a 10% formalin and 30% sucrose solution. Frozen sections were cut, and every third section was mounted onto gelled slides. Tissue was stained with neutral red to detect cannula placements. A rater, unaware of the behavioral data, assessed cannula placements. Rats with cannula tips in the most anterior portion of the BNST (+ 2.0 mm from bregma, $n = 3$), in the most posterior portion of the BNST (−1.30 mm from bregma, $n = 1$), or misplaced ventrally to the BNST ($n = 1$) or with cannulas that punctured the ventricles ($n = 4$) were excluded from the study. Rats with cannula tips centered in the BNST were included in the study (Figure 2B). This criteria resulted in 9 rats in each group for a total sample of 36 in this experiment. To determine corticosterone levels, serum was assayed using solid-phase RIA system (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) for corticosterone (assay sensitivity 5.7 ng/ml, intra-assay variability 4.3%, interassay variability 5.8%).

Results

Experiment 1

Experiment 1 assessed whether the BNST lesions prevent the stress-induced facilitation of trace eyeblink conditioning. Before training, there was no effect of BNST lesions on the numbers of baseline eyeblinks, $t(24) = 1.05$, $p > .05$, or eyeblink responses to the white noise stimulus, $t(24) = -0.67$, $p > .05$. A $2 \times 2 \times 8$ mixed-factor ANOVA was used to compare the percentage of CRs in rats that received lesion or sham surgeries and were stressed or not over 400 trials (separated into four blocks of 100 trials each) of trace conditioning; the first session was broken down into blocks of 20 trials. The ANOVA revealed an interaction between surgical manipulation and stressor exposure, $F(1, 23) = 7.07$, $p < .05$ (Figure 1C). A Newman–Keuls post hoc test indicated that stressed rats with sham surgeries emitted a greater percentage of CRs than unstressed rats with sham surgeries ($p < .05$). However, stressed rats with lesions to the BNST emitted a smaller percentage of CRs than stressed rats with sham surgeries ($p < .01$), and their responses were not different from unstressed rats exposed to a sham surgery ($p < .05$). Thus, in contrast to the stressed rats with an intact BNST, stressed rats with lesions did not emit more CRs. There was no difference in responding between the unstressed rats with and without a BNST lesion ($p < .05$), and unstressed rats with lesions emitted fewer CRs than those that were stressed after a sham surgery ($p < .01$). Although there was no three-way interaction, we were still interested in learning the time point at which differences between the groups emerged. To evaluate this, one-way ANOVAs were conducted for each block of 20 trials for the first 100 trials and

then for the blocks of 100 trials thereafter. There were no group differences until the rats had been trained for 300 trials, at which point there was a main effect of group, $F(3, 23) = 6.01$, $p < .05$. Post hoc tests revealed that rats with sham surgeries that were exposed to the stressor emitted significantly more CRs than any other group ($ps < .05$). Although it appears that the stressed rats with sham surgeries decrease in responding between 300 and 400 trials, this decrease is not significant, $t(7) = 1.99$, $p < .05$.

Experiment 2

Experiment 2 assessed whether the BNST was involved during the stressor or 24 hr later during training. BNST inactivation did not alter the baseline blink rate, $F(3, 30) = 0.44$, $p > .05$, or responses to the CS, $F(3, 31) = 2.17$, $p > .05$, before training. A 4×8 mixed-factor ANOVA analyzed the number of CRs emitted by the four groups (inactivation during stressor exposure, stress–inactivation during training, stress, no stress) over 400 trials (separated into four blocks of 100 trials each) of trace conditioning. The first 100 trials were further separated into blocks of 20 trials (see Figure 2). This analysis revealed an interaction between experimental condition and the blocks of 100 trials, $F(21, 196) = 3.07$, $p < .05$. To evaluate when the differences between these groups emerge, a one-way ANOVA was conducted with each block of 20 trials for the first 100 trials and then for the blocks of 100 trials thereafter. There were no group differences until the rats had been trained for 200 trials, at which point there was a main effect of group, $F(3, 32) = 4.69$, $p < .05$. Stressed rats infused with vehicle tended to emit more CRs than the unstressed rats, but a Newman–Keuls post hoc test revealed that the effect was not significant ($p = .08$). However, the group that received inactivation during the stressor emitted significantly more CRs than those that were unstressed ($p < .05$); in fact, this group responded similarly to those that were stressed without inactivation ($p > .05$). In contrast, rats that were stressed and then injected with muscimol during training emitted fewer CRs than those that were stressed and received vehicle infusions during training ($p > .05$). Instead, they responded similarly to the rats that were not stressed ($p > .05$). Analysis of the responses between 300 trials and 400 trials suggested a similar profile, in that the rats that received vehicle or muscimol infusions during the stressor (and vehicle during training) emitted significantly more responses than those that were stressed with BNST inactivation during training or that were left unstressed ($ps < .05$). These results indicate that inactivation of the BNST prevented the enhancement of eyeblink conditioning after stress but only when the inactivation occurred during training.

We also examined whether BNST inactivation affected the ability to emit CRs irrespective of stressor exposure. To do that, a subset of rats that had received vehicle infusions during training were exposed to an additional 100 conditioning trials in the presence of muscimol (Figure 3A). These rats were selected because they had emitted more than 40% CRs during their training in 400 trials, and thus a deficit in response to BNST inactivation could be observed. When the percentage of CRs during the last 100 trials with vehicle infusion was compared with the additional 100 trials with muscimol infusion, there was no significant decrease in performance, $t(5) = 0.45$, $p > .05$. Thus, these rats maintained their level of responding even in the presence of muscimol. This suggests that the low level of responding in the BNST inactivation group is not attributable to an inability to emit CRs. Although these data suggest that BNST inactivation does not prevent expression of the CR, it could still be argued that the low responding in the stress–inactivation during training group was because muscimol infusions prevented the acquisition of eyeblink conditioning. To address this possibility, a subset of rats that received BNST inactivation during training were trained with a more intense US (100 additional trials with a 0.65-mA US), which has been shown to increase responding. For comparison, the unstressed group, which also had low responding during training, was trained with the more intense US after vehicle infusion. A mixed-factor ANOVA compared responses between these two groups over the 100 additional trials with

the higher intensity US (separated into five blocks of 20 trials) and revealed that there was no difference in responding between the two groups, $F(1, 7) = 0.191$, $p > .05$, or interactions, $F(5, 35) = 1.85$, $p > .05$. There was a main effect of trial block, $F(5, 35) = 4.20$, $p = .004$, and a Newman–Keuls post hoc test indicated that the rats increased their responding by the third block of trials ($p < .05$). Therefore, rats that were trained in the presence of muscimol readily acquired the CR at the higher US intensity and performed at a rate comparable to unstressed controls.

A subset of rats exposed to the stressor was reexposed to the stressor at least 5 days later, and blood was collected to assess the concentrations of corticosterone. A one-way ANOVA compared corticosterone concentrations in rats with BNST inactivation during the stressor with that from rats that were stressed or unstressed without inactivation, $F(1, 2) = 17.84$, $p < .001$. Post hoc analyses indicated that concentrations in unstressed rats were less than in stressed rats in the presence of either muscimol ($p < .001$) or vehicle ($p < .001$). Groups that were stressed had similar corticosterone levels ($p > .05$) independent of whether they received muscimol or vehicle infusions. The unstressed rats had slightly higher corticosterone concentrations than unstressed rats from previous studies (Shors et al., 1999), which is most likely the result of mild stress associated with the infusions before blood collection or exposure to the anesthesia. These data indicate that BNST inactivation did not prevent the corticosterone response to the stressor.

Discussion

Exposure to an acute stressful event enhances trace eyeblink conditioning in male rats, even when training begins 24 hr after stressor exposure (Beylin & Shors, 1998; Shors, 2001). The current study examined whether the BNST is involved in this stress-induced enhancement and, if so, when. In Experiment 1, rats received permanent excitotoxic or sham lesions of the BNST. Although stressor exposure enhanced conditioning in sham controls, there was no enhancement in the BNST-lesioned group, suggesting that the BNST critically mediates the enhanced conditioning. To determine when the BNST is necessary for these effects, in Experiment 2 the BNST was inactivated either during the stressor or during training. BNST inactivation during the stressor did not prevent the stress-induced enhancement of conditioning, but BNST inactivation during training did. Further manipulations showed that the absence of a stress effect in rats with BNST inactivation during training was not due to a performance or acquisition deficit. These data indicate that the BNST is involved in the stress-induced modulation of learning and not learning itself. Together, these results suggest that neuronal activity in the BNST at the time of training critically mediates the persistent enhancement of conditioning after exposure to an acute stressful event.

As discussed, the BNST does not appear necessary during the stressor for the enhanced conditioning. Furthermore, BNST inactivation during the stressor did not prevent the corticosterone response. These results are somewhat unexpected given the projections from the BNST to hypothalamic and brainstem areas associated with the stress response (Gray, Carney, & Magnuson, 1989; Gray & Magnuson, 1987; Han & Ju, 1990; Moga, Saper, & Gray, 1989; Sawchenko & Swanson, 1983; Silverman, Hoffman, & Zimmerman, 1981; Sofroniew, 1983). Moreover, others have shown that electrical stimulation of the BNST can alter plasma corticosterone levels (Dunn, 1987), and that lesions of the BNST prevent the corticosterone response in contextual, but not cued, fear conditioning (Sullivan, Apergis, Bush, Johnson, Hou, & Le-Doux, 2004). The current data suggest that, although the BNST is in a position to affect brain regions involved in the stress response, and may do so under certain circumstances, it does not appear to be critically involved in all corticosterone responses to stressful stimuli.

Previous studies have reported that the amygdala is involved in the enhanced conditioning after stressful experience. Specifically, antagonism of NMDA receptors in the basolateral–lateral nuclei of the amygdala during the stressor prevents this effect (Shors & Mathew, 1998). However, if the antagonism occurs immediately after the stressor, enhanced learning is still observed. In contrast, the current results suggest that the BNST is not involved during the stressor but instead is necessary during training. Thus, the amygdala and BNST both appear to form part of the critical circuitry for these effects of stress on conditioning, although they are involved in different phases of the process.

Additional structures that may make up the circuitry underlying the effect of stress on conditioning include the hippocampus and the cerebellum; both structures are highly interconnected with the BNST and are necessary for trace conditioning (Beylin et al., 2001; McDonald, Shammah-Lagnado, Shi, & Davis, 1999; Solomon, Vander Schaaf, Thompson, & Weisz, 1986). The hippocampus is also sensitive to the effects of acute stressors. For example, one study has shown that exposure to a stressor that enhanced eyeblink conditioning also enhances cell excitability in area CA1 of the hippocampus (Weiss, Sametsky, Sasse, Spiess, & Disterhoft, 2005). There are also reports that stress enhances spine density in the same region (Shors, Chua, & Falduto, 2001). Because learning itself is accompanied by increases in excitability as well as spine density, this might suggest a convergence on common neuronal mechanisms (Berger, Alger, & Thompson, 1976; Gilmartin & McEchron, 2005; Leuner, Falduto, & Shors, 2003). We note, however, that the stress effect on eyeblink conditioning also occurs during training with delay conditioning, which does not require the hippocampus (Shors et al., 1991). The other brain region to consider is the cerebellum, which is required for learning the CR during both delay and trace conditioning. The BNST is connected to the cerebellar eyeblink circuit by means of direct projections to the pons (Holstege, Meiners, & Tan, 1985), which relays sensory information about the CS to the cerebellum (Christian & Thompson, 2003; Steinmetz et al., 1987; Tracey, Thompson, Krupa, & Thompson, 1998). Therefore, exposure to the stressor may alter the way in which the CS is processed during training, by means of connections between the BNST and pontine nuclei.

The BNST has been shown to be critically involved in other types of stress effects on learning processes. For example, BNST lesions prevent the standard “learned helplessness” effect. In this paradigm, animals are exposed to an inescapable or an escapable stressor followed by training on an operant conditioning task, often involving escape performance. Typically, exposure to the inescapable stressor impedes the animal’s ability to perform the escape response; however, those with lesions of the BNST do not express this deficit (Hammack, Richey, Watkins, & Maier, 2004). Also, it has been shown that lesions of the stria terminalis, which sever the afferents from the amygdala to the BNST, prevent the glucocorticoid-induced enhancement of the inhibitory avoidance response (Rooszendaal & McGaugh, 1996). Thus, it seems that the BNST may play a more general role in the effects of stress on learning. Because of this, it may be most parsimonious to suggest a general mechanism by which the BNST contributes to stress effects on learning. One such mechanism that we have considered is anxiety. A number of studies indicate that the BNST is involved in anxiety (Davis & Shi, 1999; Davis et al., 1997; Fendt et al., 2003; Lee & Davis, 1997; Walker & Davis, 1997; Walker et al., 2003). It may be that exposure to an acute stressful event induces a relatively long-lasting state of anxiety, which is mediated by the BNST. When present during the training experience, this state of anxiety may enhance an animal’s ability to form basic associative memories.

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Figure 1.

The bed nucleus of the stria terminalis (BNST) is necessary for the enhanced conditioning after stress. (A) Brain sections throughout the BNST were stained using Cajal's gold sublimite for astrocytes. This stain detects reactive gliosis associated with the excitotoxic damage from the lesion. Tissue from a sham surgery is shown to the left (4× and 20×) with cell bodies intact and without astrocytes. Tissue from a lesioned rat is shown to the right (4× and 20×). Note the absence of cell bodies and presence of darkly stained astrocytes. (B) The drawing depicts the largest lesion (in gray) and smallest lesion (in black) selected from all rats included in the study, shown here according to the atlas from Paxinos and Watson (1997). Reprinted from *The rat brain in stereotaxic coordinates* (4th ed.), by Paxinos, G., &

Watson, C. San Diego, CA: Academic Press. Copyright 1998, with permission from Elsevier. (C) Rats with BNST lesions or sham surgeries were trained with trace eyeblink conditioning after exposure to the stressor (filled circles) or after being left unstressed (open circles). Each group's percentage of CRs ($M \pm SEM$) are presented for the 400 training trials. Those with sham surgeries and exposed to the stressor emitted more conditioned responses (CRs) than their unstressed counterparts. However, rats with BNST lesions that were exposed to the stressor (filled triangles) did not respond more after the stressor and performed similarly to those that were unstressed, either with (open triangles) or without BNST lesions. These data suggest that neuronal activity within the BNST is necessary for the facilitation of trace conditioning after acute stressful experience.

A)

Group	Stressor	Training
1	Muscimol	Vehicle
2	Vehicle	Muscimol
3	Vehicle	Vehicle
4	Vehicle (No Stress)	Vehicle

B)

C)

Figure 2.

The bed nucleus of the stria terminalis (BNST) is necessary during training but not during stressor exposure for the enhanced learning that occurs after stress. The graphs represent data obtained during inactivation of the BNST as depicted in the experiment design (A). Group 1 received a muscimol infusion before stressor and vehicle (artificial cerebrospinal fluid) during training. Group 2 received vehicle infusion before stressor and muscimol infusion during training. Group 3 received vehicle infusions before stressor and before training. Group 4 received vehicle infusions the day before training but was left unstressed and received vehicle infusions during training. (B) Rats were implanted with bilateral cannulas angled at 15° to avoid the ventricles. Those placements are shown here in coronal

sections according to the atlas from Paxinos and Watson (1997). (C) The percentage of conditioned responses (CRs; $M \pm SEM$) for each condition is presented for the 400 training trials. Rats exposed to the stressor and injected with the vehicle (open circles) emitted a greater percentage of CRs than unstressed rats (filled circles), as did those with BNST inactivation during the stressor (filled triangles). However, rats with BNST inactivation during training (open triangles) did not respond more after stressor exposure. These data suggest that neuronal activity in the BNST during training is necessary for the enhanced conditioning after stressor exposure.

Figure 3.

Bed nucleus of the stria terminalis (BNST) inactivation does not disrupt trace eyeblink conditioning itself or the corticosterone response to stress. (A) Rats that received vehicle infusions during training and later received muscimol infusions during training continued to emit conditioned responses (CRs) despite BNST inactivation. (B) Rats with BNST inactivation during training (open triangles) with a high-intensity unconditioned stimulus (US) rapidly acquired the CR and responded similarly to unstressed rats without BNST inactivation (filled circles); that is, both groups acquired the task when the US was more intense. (C) Rats with BNST inactivation during the stressor exposure (striped bar) continue to release corticosterone (CORT), and their levels were not different from those stressed rats infused with the vehicle (white bar). Concentrations were elevated in both groups compared with concentrations in the unstressed controls (black bar).