

LESIONS IN THE BED NUCLEUS OF THE STRIA TERMINALIS DISRUPT CORTICOSTERONE AND FREEZING RESPONSES ELICITED BY A CONTEXTUAL BUT NOT BY A SPECIFIC CUE-CONDITIONED FEAR STIMULUS

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Abstract—The bed nucleus of the stria terminalis (BNST) is believed to be a critical relay between the central nucleus of the amygdala (CE) and the paraventricular nucleus of the hypothalamus in the control of hypothalamic–pituitary–adrenal (HPA) responses elicited by conditioned fear stimuli. If correct, lesions of CE or BNST should block expression of HPA responses elicited by either a specific conditioned fear cue or a conditioned context. To test this, rats were subjected to cued (tone) or contextual classical fear conditioning. Two days later, electrolytic or sham lesions were placed in CE or BNST. After 5 days, the rats were tested for both behavioral (freezing) and neuroendocrine (corticosterone) responses to tone or contextual cues. CE lesions attenuated conditioned freezing and corticosterone responses to both tone and context. In contrast, BNST lesions attenuated these responses to contextual but not tone stimuli. These results suggest CE is indeed an essential output of the amygdala for the expression of conditioned fear responses, including HPA responses, regardless of the nature of the conditioned stimulus. However, because lesions of BNST only affected behavioral and endocrine responses to contextual stimuli, the results do not support the notion that BNST is critical for HPA responses elicited by conditioned fear stimuli in general. Instead, the BNST may be essential specifically for contextual conditioned fear responses, including both behavioral and HPA responses, by virtue of its connections with the hippocampus, a structure essential to contextual conditioning. The results are also not consistent with the hypothesis that BNST is only involved in unconditioned aspects of fear and anxiety. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: Pavlovian, amygdala, bed nucleus of the stria terminalis, hypothalamic–pituitary–adrenal (HPA) axis, lesion, glucocorticoids.

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Abbreviations: ANOVA, analysis of variance; AP, anteroposterior; BNST, bed nucleus of the stria terminalis; CE, central nucleus of the amygdala; CORT, corticosterone; CS, conditioned stimulus; DV, dorsoventral; HPA, hypothalamic–pituitary–adrenal; LSD, least significant difference; ML, mediolateral; PVN, paraventricular nucleus of the hypothalamus; US, unconditioned stimulus.

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Activation of the hypothalamic–pituitary–adrenal (HPA) axis, including release of glucocorticoids, is a central component of the adaptive response to real or anticipated aversive physical or psychological challenge. Surprisingly little is known about the neural circuits by which environmental stimuli come to elicit HPA responses. Fear conditioning, a behavioral model of emotional stress, is potentially useful for exploring this issue since the neural pathways by which stimuli initiate fear behaviors and associated autonomic responses have been characterized in detail (LeDoux, 2000; Davis and Whalen, 2001; Maren, 2001).

Through fear conditioning an organism learns that a simple sensory stimulus (a cue), or more complex environmental representation (a context), predicts imminent adversity. The neural pathways that mediate the acquisition and expression of conditioned fear crucially involve the amygdala. Incoming stimuli are processed by the lateral nucleus of the amygdala, which, by way of direct and indirect intra-amygdala connections, activate the central nucleus of the amygdala (CE). CE, in turn, connects with areas involved in the expression of aversive responses. Thus, while damage to CE disrupts the expression of a broad spectrum of conditioned fear responses, including HPA responses, localized damage to areas that CE projects to affect only individual components of the fear response mediated by the particular region (Davis, 1992; LeDoux, 1996, 2000; Maren and Fanselow, 1996).

The point of origin in the brain of the HPA axis is the paraventricular nucleus of the hypothalamus (PVN; Guillemin and Rosenberg, 1955; Saffran et al., 1955). CE has direct, albeit relatively sparse, projections to PVN (Gray et al., 1989; Prewitt and Herman, 1998). In addition, CE projects to the bed nucleus of the stria terminalis (BNST), which, in turn, has dense projections to PVN (Swanson, 1987). Previous studies have found that lesions of the BNST attenuate the release of glucocorticoid hormone (corticosterone, CORT) in response to conditioned fear stimuli (Gray et al., 1993). These anatomical and neuroendocrine findings have led to the view that connections from CE to BNST and, in series, connections from BNST to PVN, mediate HPA responses elicited by conditioned fear stimuli (LeDoux, 1996, 2000).

If the BNST is the primary link between CE and PVN, lesions of BNST should block the expression of HPA responses regardless of whether those responses are elicited by a specific cue stimulus or a contextual stimulus.

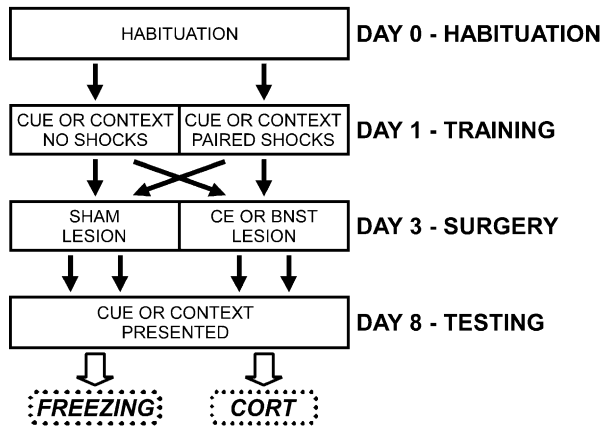


Fig. 1. Schematic of general procedures carried out for each of the four experimental components.

Studies performed to date have used either contextual stimuli alone, or a combination of contextual stimuli and a specific cue stimulus, and therefore do not address the question of whether BNST lesions block HPA responses elicited by specific cues (Van de Kar et al., 1991; Gray et al., 1993; Goldstein et al., 1996). In the present study we therefore assessed the effects of lesions of CE or BNST on the HPA neuroendocrine (CORT) response and behavioral response (freezing) elicited by either a conditioned cue (a tone) or a conditioned context. The lesions were made *after* training to isolate the effects of localized brain damage on the *expression* of the responses, avoiding the potentially confounding effects of lesion on acquisition of fear memory.

EXPERIMENTAL PROCEDURES

Subjects

Adult male Sprague–Dawley rats (Hilltop Laboratories, Scottsdale, PA, USA) weighing 275–325 g at the start of the experiments were used. All animals were acclimated for 2 weeks prior to experimentation. Animals were housed individually in plastic Nalgene cages (Nalge Nunc International, Rochester, NY, USA). The animal colony was on a 12-h light/dark cycle (lights on at 08:00 h) with food and water provided *ad libitum*. All animals utilized were behaviorally naive. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Experimental Animals and with approval by the New York University animal care and use committee. The number of animals utilized was kept to the minimum necessary to allow for statistically meaningful evaluation of the outcome data. Every effort was made to minimize any suffering or discomfort of the animals.

Overall design

The experiment had four components in order to test each of the four experimental conditions of interest. They were: 1) CE lesion/sham and testing for conditioned cue response; 2) CE lesions/sham and testing for conditioned contextual response; 3) BNST lesion/sham and testing for conditioned cue response; and 4) BNST lesion/sham and testing for conditioned contextual response. See Fig. 1 for the general procedures carried out for each experimental component.

Surgery

The rats were randomly assigned to “sham” and “lesion” groups for the two target brain regions, CE and BNST. Surgery took place 2 days after classical conditioning and 5 days before behavioral testing, as described below.

CE lesions. The rats were anesthetized with 100 mg/kg i.p. of ketamine HCl (Ketaset; Phoenix Pharmaceutical, St. Joseph, MO, USA) and placed into a stereotaxic frame using blunt ear bars to protect the tympanic membranes. Flat skull coordinates are given in millimeters, obtained from Paxinos and Watson (1997). The anteroposterior (AP), mediolateral (ML), and dorsoventral (DV) coordinates were referenced from bregma. CE lesions were made in three locations bilaterally: (1) AP=−1.8 mm, ML=±4.4 mm, DV=−8.4 mm to skull surface; (2) AP=−2.3 mm, ML=±4.4 mm, DV=−8.4 mm to skull surface; and (3) AP=−2.8 mm, ML=±4.4 mm, DV=−8.4 mm to skull surface. All animals had six burr holes placed in the skull, carefully exposing but not damaging the dura mater at the three sites per ML side. For animals in lesion groups, small bilateral electrolytic lesions were prepared for each selected coordinate as follows: a small incision was made in the dura; a monopolar electrode (RH NE-100 01×50 mm, David Kopf Instruments, Tujunga, CA, USA), insulated with epoxy to within 200 μm of the tip, was lowered to each selected coordinate; 60 s was allowed to pass; positive current (0.5 mA) was passed for 13 s; and the electrode was removed. Sham groups were treated identically to the lesion groups except that coordinates of the electrode tips were 1.0 mm dorsal (i.e. −7.4 mm DV) to the lesion coordinates and no current was passed. After completion of surgery the wound was closed, ketamine anesthesia was discontinued, one dose of 0.3 mg/kg s.c. of buprenorphine HCl (Henry Schein Inc., Melville, NY, USA) was administered, and the animal recovered under a heat lamp until mobilizing.

BNST lesions. The surgical procedure was identical to that for CE lesions except for the following differences. Coordinates for the three lesions per side in were: (1) AP=0.0 mm, ML=±1.5 mm, DV=−7.0 mm; (2) AP=−0.5 mm, ML=±1.5 mm, DV=−7.0 mm; and (3) AP=−1.0 mm, ML=±1.5 mm, DV=−7.0 mm. Current was passed for 16 s for each of the six lesion sites. Sham groups had electrode tips 1.0 mm dorsal (i.e. −6.0 mm DV) to the coordinates of the lesion groups and no current was passed.

Classical fear conditioning

Apparatus. Prior to each phase of the experiment, animals were transported to a holding room in the vivarium that was distinct from the rooms in which behavioral procedures took place. The chambers used for conditioning and testing were of two types, each housed in sound attenuated cubicles, “Chamber A” (Coulbourn Instruments, Lehigh Valley, PA, USA; model E10-10 and model E10-20) and “Chamber B” (Med Associates, Inc Georgia, VT, USA; model ENV-001 and model ENV-022M). The floors consisted of metal bars for delivery of footshock. In certain conditions, described below, a black plastic floor was inserted in chamber B such that it covered the metal bars. For behavioral procedures involving conditioning and testing to a specific cue, both chambers were utilized. For conditioning and testing to a context, only chamber B was utilized. Chambers A and B, including floors, were cleaned before and after utilization. Chamber B was also cleaned with a mild peppermint-scented soap.

Classical conditioning procedures. Rats were subjected to either *cue conditioning*, in which a tone conditioned stimulus (CS) was paired with a footshock unconditioned stimulus (US) or *context conditioning*, in which the US was delivered in the conditioning apparatus in the same manner as in cue conditioning but in the absence of an explicit cue CS.

Cue conditioning. On day 0 (Habituation Day), the rats were acclimated to the conditioning chamber (chamber A) and the testing chamber (chamber B, black plastic floor inserted) for 20 min each and then returned to the home cage. The rats were then divided into two groups for subsequent training, one for conditioning and the other for a “no-shock” control group. On day 1 (Conditioning Day), between 12:00 h and 15:00 h, animals in the cue conditioned group received five presentations of a tone-shock pairing in which the tone (5 kHz, 76 dB, 20 s) co-terminated with a footshock (1.0 mA, 500 ms). A computer generated random inter-trial intervals between presentations of the shock with a mean inter-trial interval of 120 s. The no-shock group was treated identically except that the shocker did not deliver current. All animals were in the conditioning chamber for 15 min.

Context conditioning. This procedure was identical to cue conditioning except that the same chamber (chamber B, no plastic floor inserted) was utilized for conditioning as would be subsequently used for testing, and the tone CS was omitted during conditioning and testing. Therefore on day 0 (Habituation Day) the animals were habituated for 20 min to chamber B only; On day 1 (Conditioning Day), between 12:00 h and 15:00 h, animals in the context conditioned group received five presentations of a footshock (1.0 mA, 500 ms). A computer generated random inter-trial intervals between presentations of the shock with a mean inter-trial interval of 120 s. The no-shock control group was treated identically except the shocker did not deliver current. All animals were in the conditioning chamber for 15 min.

Behavioral testing procedures. On day 3 (Surgery Day), the animals were operated on for the purpose of placing lesions in the CE or BNST, as described above. On day 8 (Testing Day), after 5 days of recovery from surgery, between 10:00 h and 12:00 h all animals were tested in chamber B. Behavior was recorded by a microcamera (mounted in a 1.5-inch hole in the ceiling of the chamber) for scoring of freezing responses at a later time. Freezing was defined as immobility, with the exception of respiratory-related movement, in the stereotyped crouching posture (Blanchard and Blanchard, 1969; Fanselow, 1980). The total time spent freezing during the respective sampling periods for cue conditioning and context conditioning, described below, was scored by an observer who was blind to group assignment.

Cue conditioning. Chamber B was distinct from chamber A in structure, scent, and lighting. The insertion of black plastic floors into chamber B for the cue conditioning component further differentiated the context from chamber A. Previous studies in the laboratory have shown that the use of these specific contextually distinct environments virtually isolates conditioned response to cue stimuli (Sullivan et al., 2003). Animals each received five presentations of the tone CS (average inter-trial interval of 120 s) alone (no US). Means were calculated from the percent of time each animal spent freezing during the total periods of each of five presentations of the 20 s tones, and this served as the behavioral outcome measure. The total time spent in the testing chamber was 12 min for all animals. Next, each was placed back in its respective home cage which was on a cart in a separate sound attenuated holding room.

Context conditioning. On day 8 (Testing Day), the animals were placed in chamber B (with identical contextual stimuli as were present during training including no plastic floor inserted). Freezing behavior was measured by scoring the percent of time freezing for the duration of every 30 s period of the last 30 s of each minute of the first 5 min (i.e. five samples of 30 s each were assessed for every other 30 s interval of the 5 min starting at time=30 s in chamber); the mean percent of time freezing during each of the five 30 s periods was calculated. The total time spent in the testing chamber was 6 min for all animals. Next, each was

placed back in its respective home cage which was on a cart in a separate sound attenuated holding room.

Blood collection and CORT analysis

Twenty minutes after exposure to the first tone (cue conditioning procedure) or the context (context conditioning procedure) each animal was taken immediately to a separate room and decapitated rapidly. Trunk blood was recovered and centrifuged for 15 min in a microfuge, and separated serum was collected and stored at -70°C until the CORT assay. Circulating CORT (ng/ml) was measured in duplicate using the solid-phase radioimmunoassay system specific for rat CORT (“Coat-A-Count”; Diagnostic Products Laboratory, Los Angeles, CA, USA). Assay sensitivity was 20 ng/ml, and intra-assay and inter-assay variability was less than 4% and less than 6%, respectively.

Histological analysis

After collection of trunk blood, brains were removed and placed in 10% formalin. Following immersion for at least 2 weeks, the brains were frozen and sectioned (50 μm) through the relevant brain areas using a cryostat. Every section was mounted onto gelatin-coated slides and stained with Cresyl Violet. Electrolytic lesions were evaluated according to the location and extent of the tissue damage by an observer who was blind to corresponding CORT and behavioral data. Data were only utilized for lesioned animals with bilateral damage to the area of interest with minimal infringement on neighboring nuclei, as described below.

Statistical analysis

All analyses were conducted using Statistica '98 Edition Release 5.1 (StatSoft, Inc., Tulsa, OK, USA). The two outcome measures, freezing behavior and CORT response, were separately analyzed. Each analysis was completed using a two-way analysis of variance (ANOVA) to test for significant effects of lesion (CE lesion, BNST lesion, Sham), stimulus (cue, context), and lesion-by-stimulus interaction. Percent freezing was the dependent variable for the behavioral analysis, performed on data from rats in the fear conditioned groups. For the neuroendocrine analysis, plasma CORT responses for rats in the conditioned groups were represented as a percentage of the mean CORT response for the corresponding no-shock control group. Significant interactions were broken down with a simple effects analysis for lesion at each stimulus type, and then mean comparisons were performed using Fisher's post hoc least significant difference (LSD) *t*-tests. Values are expressed as means \pm S.E.M.

RESULTS

Effect of post-training lesions of the CE or BNST on behavioral and CORT response to cue and context conditioning

Histological results. Of the 71 CE lesioned animals, 30 met anatomical and histological criteria. Damage to the CE was sustained to its full rostral–caudal extent, and included the lateral CE as well as significant portions of the medial and capsular parts of the CE. The commissural stria terminalis was also damaged in some rats. The borders of the CE lesions were the basal amygdala on the lateral side and the lateral globus pallidus on the medial side, with minimal infringement on either side.

Of the 68 BNST lesioned animals, 33 met anatomical and histological criteria. Damage to the BNST included nearly the full extent of the lateral division of the BNST, occasionally excluding the most lateral portion of the jux-

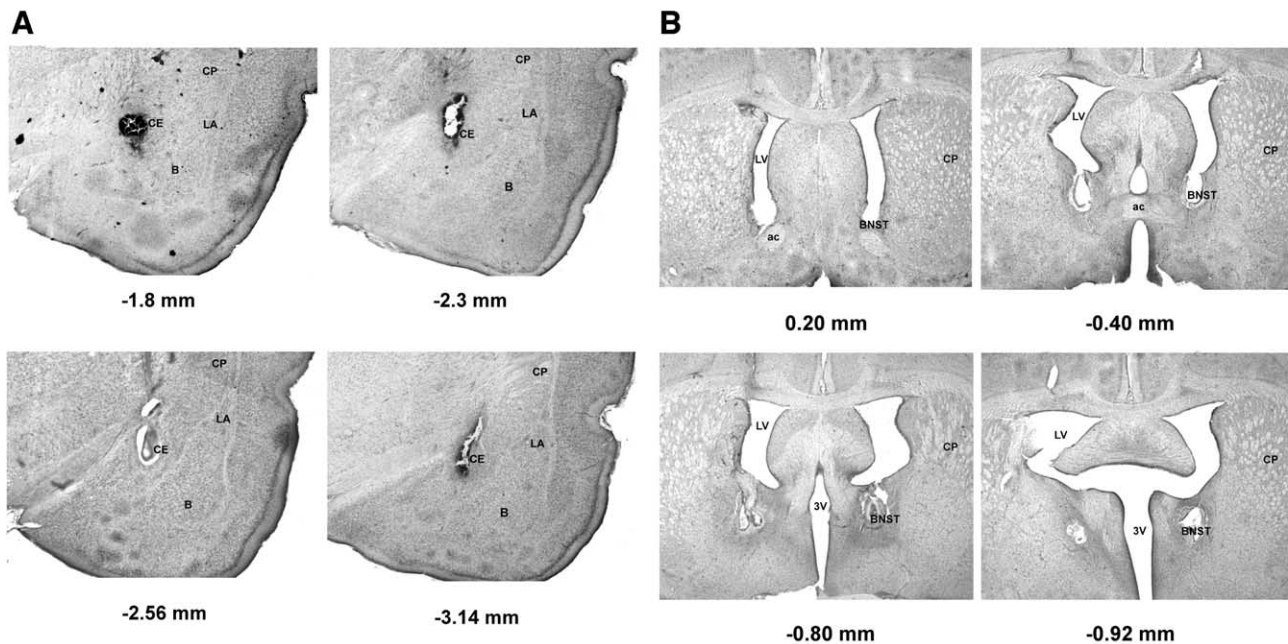


Fig. 2. Photomicrographs showing typical lesions of (A) the CE, and (B) the BNST. Numbers represent millimeters from bregma in the AP plane. ac, anterior commissure; B, basal nucleus of the amygdala; LA, lateral nucleus of the amygdala; CP, caudate putamen; LV, lateral ventricle; 3V, third ventricle.

tacapsular part that was closest to the internal capsule, and the most anterolateral portion of the lateral BNST that lies on the caudal border of the nucleus accumbens. In addition, there was extensive damage to the anterior part of the medial division of the BNST, excluding the anterior part medial to the stria terminalis just below the lateral septum. Substantial (but not complete) damage to the posteromedial part of the medial BNST was also evident. Substantial damage to the posterolateral and posterointermediate parts of the medial BNST was evident in some, but not all, rats. Little to no damage was sustained to fiber tracts in the region, such as the medial forebrain bundle and the internal capsule, with the exception of the stria terminalis, which was ablated along with all BNST lesions, and small portions of the posterior part of the anterior commissure.

Behavioral and CORT responses did not differ between animals with sham CE and BNST lesions, and so the data from rats with CE and BNST sham lesions from each stimulus and training condition were collapsed for all subsequent analyses. See Fig. 2 for representative lesions and Fig. 3 for a depiction of the smallest and largest electrolytic lesions.

Behavioral results. Rats from all no-shock control groups showed no freezing to tone or context (data not shown). In fear conditioned rats, two-way ANOVA with freezing as the dependent variable demonstrated significant effects for lesion, $F(2,74)=43.14$, $P<0.001$; stimulus, $F(1,74)=38.13$, $P<0.001$; and lesion by stimulus interaction, $F(2,74)=6.33$, $P=0.003$. Simple effects for lesion were significant for both cue, $F(2,74)=28.63$, $P<0.001$; and context, $F(2,74)=20.58$, $P<0.001$. Post hoc LSD t -

tests for cue conditioning showed that CE lesions caused a significant impairment in freezing relative to sham-lesioned controls ($P<0.001$), but that BNST lesions did not. Post hoc LSD t -tests for context conditioning showed that both CE lesions and BNST lesions caused a significant impairment in freezing relative to sham-lesioned controls ($P<0.001$). Means (\pm S.E.M.) and comparisons of interest for the behavioral data are presented graphically in Fig. 4A.

CORT results. With rats in the no-shock groups, CORT levels were not significantly different between lesion groups (sham, CE, BNST) exposed to the same stimulus conditions (cue or context). Thus, CORT levels from controls were collapsed across lesion groups separately for each stimulus condition, and then averaged to serve as a baseline for calculating CORT increases produced by fear stimuli (cue or context). The resulting percent baseline scores were used for the following analysis. Two-way ANOVA with percent baseline CORT as the dependent variable demonstrated significant effects for lesion, $F(2,74)=17.82$, $P<0.001$; and lesion by stimulus interaction, $F(2,74)=4.98$, $P=0.009$; while the effect for stimulus type showed only a trend toward significance, $F(1,74)=3.48$, $P=0.066$. Simple effects for lesion were significant for both tone, $F(2,74)=18.44$, $P<0.001$; and context, $F(2,74)=5.13$, $P=0.009$. Post hoc LSD t -tests for cue conditioning showed that CE lesions caused a significant reduction in CORT levels relative to sham-lesioned controls ($P<0.001$), but that BNST lesions did not. Post hoc LSD t -tests for context conditioning showed that both CE lesions and BNST lesions caused a significant reduction in CORT levels relative to sham-lesioned controls ($P=0.01$). Means

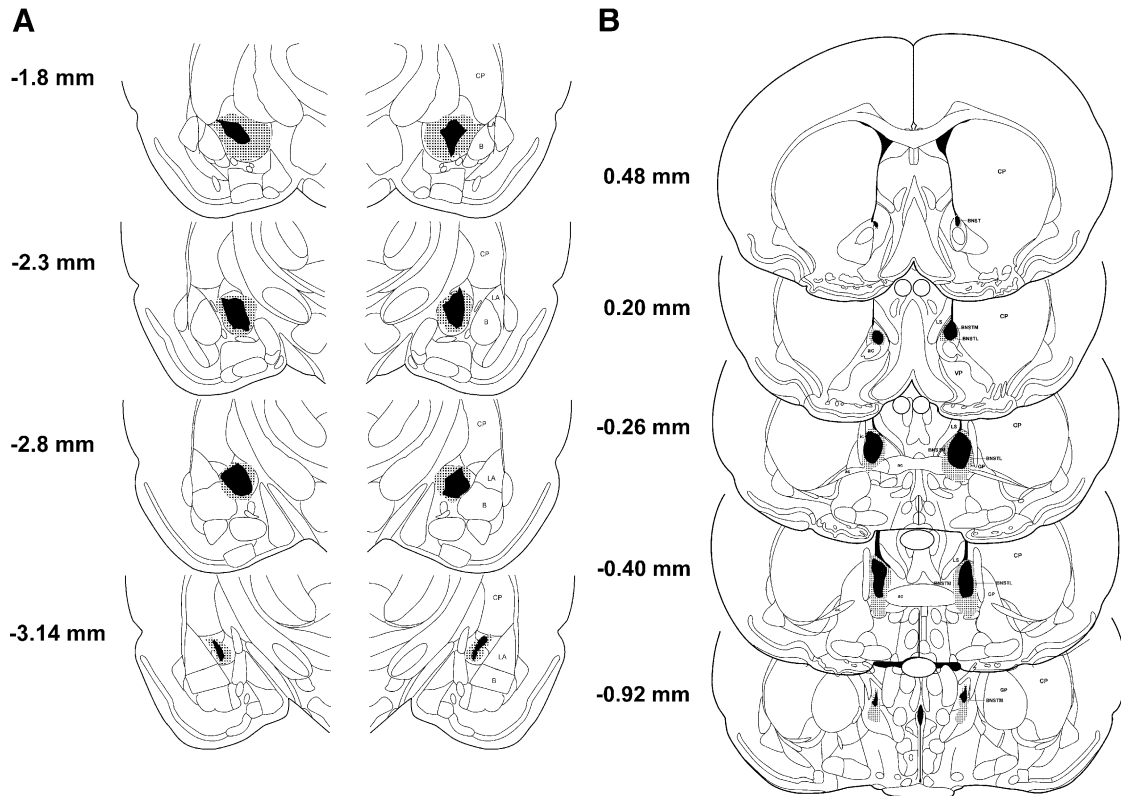


Fig. 3. Reconstruction of smallest (black shading) and largest (gray shading) electrolytic lesions of (A) the CE, and (B) the BNST. Numbers represent millimeters from bregma in the AP plane. Diagrams were adapted from CD-ROM accompanying Paxinos and Watson (1997) atlas. ac, anterior commissure; B, basal nucleus of the amygdala; BNSTL, BNST, lateral division; BNSTM, BNST, medial division; LA, lateral nucleus of the amygdala; CP, caudate putamen; GP, globus pallidus; ic, internal capsule; LS, lateral septum; VP, ventral pallidum.

(\pm S.E.M.) and comparisons of interest for the behavioral data are presented graphically in Fig. 4B.

DISCUSSION

The neural pathways through which emotionally salient environmental events initiate HPA stress hormone responses are poorly understood. In this study, we pursued this issue using classical fear conditioning, a form of emotional stress that has been studied extensively at the neural level. We focused on the role of two structures previously implicated in the activation of the HPA axis by conditioned fear stimuli, the CE and the BNST, and compared the effects of electrolytic lesions of these structures on the expression of HPA axis neuroendocrine responses (serum CORT responses) and conditioned fear behavior (freezing responses) elicited by a specific conditioned fear stimulus (a tone CS previously paired with footshock) or a diffuse contextual stimulus (the chamber in which footshock was previously presented in the absence of any specific cue CS). Our results showed that bilateral electrolytic lesions of CE blocked both freezing and CORT responses regardless of whether a tone or contextual CS was used. In contrast, BNST lesions blocked CORT and freezing responses to contextual stimuli but not to a discrete tone CS.

Considerable evidence supports the view that the CE is a key structure in the expression of behavioral, autonomic, and

neuroendocrine conditioned fear responses elicited by both specific cues and contextual stimuli (Kapp et al., 1992; LeDoux, 1992, 2000; Maren, 2001). Specifically, previous studies have found that CE lesions block both behavioral and neuroendocrine responses elicited by contextual stimuli (Van de Kar et al., 1991; Roozendaal et al., 1992; Maren and Fanselow, 1996), as well as behavioral and autonomic responses elicited by a tone CS (Kapp et al., 1979; Hitchcock and Davis, 1986; Iwata et al., 1986; LeDoux et al., 1988). Although some researchers have emphasized a role for CE in the acquisition, rather than expression, of contextual fear-induced CORT responses (Roozendaal et al., 1992), the use of a post-lesion re-training session in this study complicated the distinction between acquisition and expression. Consequently, we focused on the role of CE in fear expression, and trained all rats before lesions were made. Furthermore, because no prior study had examined freezing and HPA axis responses elicited by both tone and contextual stimuli, we did so. Our results showing that damage to CE blocks both CORT response and freezing to the two classes of stimuli are consistent with the hypothesis that CE is an obligatory part of the circuitry through which both freezing and HPA conditioned responses are elicited, regardless of the type of CS. CE has also been implicated in adrenocortical responses to other (Beaulieu et al., 1986, 1989; Roozendaal et al., 1991; Van de Kar et

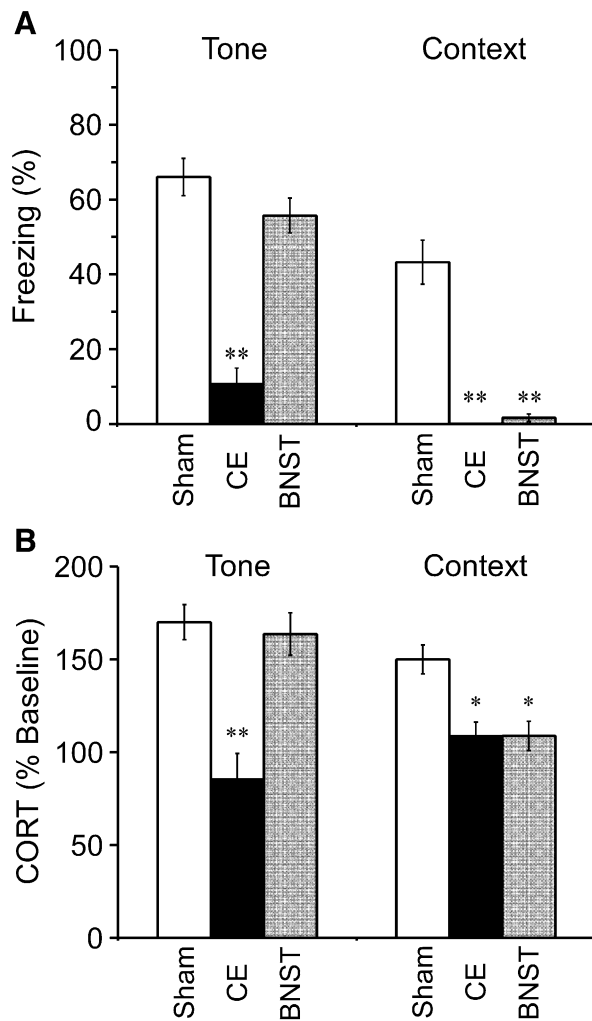


Fig. 4. Effects of post-training lesions on testing response. (A) Effects of CE or BNST lesions on cue- and context-evoked freezing. (B) Effects of CE or BNST lesions on cue- and context-evoked increases in CORT, expressed as the percent of baseline CORT from controls. Bar values are mean \pm S.E.M. Asterisks represent level of significance (* $P < 0.05$; ** $P < 0.01$) indicated by post hoc mean comparisons using Fisher's LSD test.

al., 1991), although not all (Marcilhac and Siaud, 1996; Prewitt and Herman, 1997), forms of stress.

Because we used electrolytic lesions, it is possible that the effects we found for CE lesions were due to damaged fibers passing through but not terminating in CE. However, previous studies found that CORT responses elicited by contextual stimuli were disrupted by neurotoxic lesions of CE which spared fibers of passage (Van de Kar et al., 1991). Still, in that study lesions were made prior to training, which complicates the interpretation of the distinct role of the CE in the acquisition versus the expression of conditioned fear responses. A separate study found that post-training neurotoxic lesions of the amygdala abolished freezing and CORT responses to tone and contextual stimuli presented together at the same time, which precludes any conclusion about the role of amygdala regions in processing each of the two classes of stimuli (Goldstein et al.,

1996). Also, the lesions in that study were more extensive and appeared to include CE as well as the basal and accessory basal nuclei of amygdala. When those results are considered with work showing that electrolytic lesions of the basal and accessory basal amygdala have no effect on the freezing elicited by a tone CS (Nader et al., 2001), it suggests that the effects of the large post-training neurotoxic lesions in the area of CE, at least on tone elicited responses, were the result of damage to CE. Generalizing across all these studies, the most parsimonious explanation of the effects we found here with discrete electrolytic lesions to CE is that the CE is an obligatory structure in the control of behavioral, autonomic, and HPA axis responses elicited by conditioned fear stimuli.

In contrast to CE lesions, post-training bilateral destruction of BNST did not significantly disrupt the behavioral and CORT responses elicited by the tone CS. These electrolytic lesions encompassed virtually the full AP extent of BNST, excluding only the most posterior regions. Thus, the entire lateral division and most of the medial division were damaged. Therefore the lack of effect on tone-elicited responses indicates that neither BNST itself nor fibers passing through it play a significant role in the CORT or behavioral responses to a tone CS. This is consistent with prior work showing that *pretraining* bilateral ibotenic acid lesion of BNST had no effect on *autonomic* or freezing responses elicited by a tone CS (LeDoux et al., 1988). It should also be noted that our BNST lesions included ablation of the stria terminalis, so it can also be concluded that the stria terminalis is not required for conditioned fear-evoked CORT response by discrete auditory cues. Our present findings, taken with past work, therefore suggest that neither the BNST nor the stria terminalis plays a role in the constellation of fear responses (including behavioral, CORT, and autonomic responses) elicited by a tone CS.

Previous studies (Gray et al., 1993) using only a contextual CS, have led to the general notion that the BNST is a crucial link between the CE and the PVN for conditioned fear-induced HPA axis response (Herman and Cullinan, 1997; Van de Kar and Blair, 1999). However, our results suggest a more specific role of BNST in conditioned fear-induced HPA activation in that it specifically mediates CORT responses to contextual stimuli. Additionally, we observed that BNST lesions disrupted the freezing response to the context, while they did not disrupt this behavioral response to the tone. Therefore, our results suggest that the previously observed effect of BNST lesions specifically on conditioned HPA response may in fact be due to a more general role of BNST in processing and control of conditioned responses to contextual cues, both glucocorticoid *and* behavioral. This is consistent with anatomical data indicating dense, topographically organized connections between the subicular region of hippocampal formation, a region known to be essential to contextual processing (Kim and Fanselow, 1992; Phillips and LeDoux, 1992), and the BNST (Swanson and Cowan, 1977; Cullinan et al., 1993; Dong et al., 2001).

It must be acknowledged that the extensive electrolytic lesions in the BNST region, although not obviously disrupting

the known pathway between CE and periaqueductal gray (Hopkins and Holstege, 1978; Rizvi et al., 1991) that mediates freezing behavior, may have disrupted a region, or essential fibers of passage, involved in the conditioned freezing response. Arguing against this point, lesions created in the same manner for the tone component of our experiment did not have a significant effect on freezing behavior.

Together, these findings suggest that the efferents from CE that mediate the HPA axis response to a tone CS involve the direct, yet sparse, pathway from CE to PVN (Gray et al., 1989; Prewitt and Herman, 1998) and/or an alternate pathway that remains to be elucidated. There is some evidence that monoaminergic systems play a role in this type of response. For example, it has been shown that lesions of CE produce a dramatic attenuation of plasma adrenocorticotropin hormone response to immobilization stress at the same time as resulting in a change in baseline and stress-induced serotonergic activity at the levels of hypothalamus and amygdala (Beaulieu et al., 1986). Similarly, the noradrenergic and dopaminergic systems appear to be involved in CE-mediated activation of the HPA axis in response to immobilization stress (Beaulieu et al., 1987). However, it must be kept in mind that these studies involved an unconditioned stressor (e.g. immobilization), and there is conflicting evidence for the role of the amygdala in the stress responses to such stimuli (Prewitt and Herman, 1997).

Our results also have relevance to the hypothesis that BNST plays an especially important role in unconditioned fear and anxiety (Walker and Davis, 1997; Fendt et al., 2003). For example, BNST lesions disrupt a rat's spontaneous preference for a dark over a light chamber, a response generally believed not to involve conditioning (Walker and Davis, 1997). This type of response is essentially an unconditioned form of contextual fear, thus supporting our speculation that BNST is especially involved in processing contextual stimuli. However, other studies have found that unconditioned freezing responses to aversive tones (Schulz and Canbeyli, 1999) or to trimethylthiazoline (Fendt et al., 2003), a component of fox feces odor, are also affected by damage to BNST. These results are not obviously related to contextual processing. However, it is well established that presentation of an aversive US results in rapid contextual conditioning. Indeed, freezing to shocks and other unconditioned stimuli has often been interpreted as due to the elicitation of *conditioned fear* by the context rather than to the unconditioned effects of the aversive stimulus (Fanselow, 1980). It is thus possible that contextual processing accounts for the effects of BNST lesions in these studies, but additional experiments would be necessary to test this. At this point, the most conservative conclusion is that BNST is involved in contextual processing of threats, as well as certain aspects of unconditioned fear.

The dense projections of BNST to PVN have been described (Swanson and Sawchenko, 1980; Sawchenko and Swanson, 1983), and Gray et al. (1993) emphasized that the afferent and efferent pathways of the *lateral* BNST show striking similarities to those of CE, having bidirectional connections with brainstem regions such as central gray, parabrachial nucleus, and the dorsal vagal complex (Gray et al., 1993). Yet electrical stimulation studies indi-

cate *medial* BNST plays a role in stimulation of CORT as well as a pressor effect on blood pressure, whereas stimulation of lateral BNST results in decreased plasma CORT and depressor activity on blood pressure (Dunn, 1987; Dunn and Williams, 1995). Our BNST lesions encompassed much of both the medial and lateral divisions and therefore did not differentiate the role of each division. If the ibotenic acid lesions of Gray et al. (1993) were specific to the lateral division, the effect of lesion we observed in the attenuation of CORT response may have been mediated by tissue damage in this division. On the other hand, the injection site in that study was only 0.2 mm lateral to our electrolytic lesion coordinates, and it is not clear if the lesions impinged on the medial division to some extent. Future work needs to address the effects of lesioning specific BNST divisions on behavioral, autonomic, and neuroendocrine response to conditioned contextual stimuli. In addition, use of post-training excitotoxic lesions are necessary to confirm that the observed effects of CE lesions on tone and contextual HPA responses are due to destruction of local neurons and not to destruction of fibers of passage. It will also be of interest to see if there is a similar effect of BNST lesion on autonomic response to a conditioned context as we observed for behavioral and CORT response, exploring a broader role for BNST in fear response to contextual information.

In summary, our results help to clarify the role of the BNST in conditioned fear, questioning both the view that BNST is a critical link between the CE and the PVN in the control of HPA responses to all forms of fear stimuli, and the notion that BNST is mainly involved in unconditioned fear and anxiety. Our results instead suggest that BNST is mainly involved in fear responses to contextual stimuli, including both behavioral and endocrine responses elicited by learned and unlearned situations.

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