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Role of the Bed Nucleus of the Stria Terminalis in the Acquisition and Expression of Conditioned Defeat in Syrian Hamsters

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Abstract

When Syrian hamsters (*Mesocricetus auratus*) are defeated by a larger, more aggressive opponent, they subsequently produce more defensive and submissive behaviors and less chemosensory investigation and aggression, even when they are paired with a smaller, non-aggressive intruder. This persistent change in the behavior of defeated animals has been termed conditioned defeat. In the present study, we tested the hypothesis that the bed nucleus of the stria terminalis (BNST) is important for the acquisition and expression of conditioned defeat. We found that the GABA_A receptor agonist muscimol infused into the BNST immediately prior to initial defeat training failed to disrupt the acquisition of conditioned defeat, while muscimol infused prior to testing caused a significant reduction in submissive/defensive behaviors and an increase in investigatory behaviors of the non-aggressive intruder. These results indicate that 1) the BNST, unlike the amygdala, does not appear to be critically involved in the consolidation process related to the memory of social defeat and 2) the BNST may be an important site for the execution of fear behaviors associated with social defeat. Considering the high degree of connectivity between the BNST and the amygdala, these findings provide further insight into the neural circuitry governing conditioned defeat and support the view of a functional dissociation between the amygdala and the BNST in the modulation of conditioned fear in an ethologically relevant model.

Keywords

Social defeat; Conditioned Fear; Anxiety; Stress; Defensive Behavior

Introduction

Agonistic interactions among members of the same species are a common, yet very potent, form of stress. These interactions typically results in a 'winner' and a 'loser', and losing has been shown to have behavioral and physiological consequences. For example, defeated animals consistently show a general increase in defensive behaviors, including freezing and risk assessment, while exhibiting reduced aggressive and pro-social behaviors [2,3]. Social stress can also cause changes in brain morphology, including reduced branching and total dendritic length of the apical dendrites in the CA3 pyramidal neurons of the hippocampus [17] as well as more peripheral changes such as increased cortisol secretion [2,3,10] and a concomitant reduction in circulating levels of testosterone [3,10,20]. The long-term consequences of social

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defeat can therefore have severe debilitating effects on an organism [4,5] and may result in irreversible and widespread systemic changes.

Because Syrian hamsters are territorial, they readily and reliably exhibit agonistic behaviors, even in a laboratory environment, when an intruder is placed into the home cage of another hamster. Despite the high incidence of agonistic behavior, hamsters are very rarely injured during brief interactions. These characteristics make hamsters an ideal species with which to study the consequences of social defeat. Over the past several years, our laboratory has employed an ethological model of conditioned fear called *conditioned defeat* [19]. Briefly, a hamster is placed into the home cage of a larger, more aggressive animal ("resident aggressor", or RA) wherein it is quickly defeated (usually within one minute). When the defeated animal is subsequently exposed in its own home cage to a non-aggressive intruder (NAI), it exhibits high levels of defensive and submissive behaviors and a lack of chemosensory investigation and aggression. In contrast, a weight and age-matched hamster that has not been recently defeated reliably attacks and defeats a NAI.

Using the conditioned defeat model, we are beginning to identify the components of a closely linked circuit of structures, including various sub-nuclei of the amygdala, that are responsible for the acquisition and expression of conditioned defeat [11,12,16]. Our findings are consistent with the large number of studies [1,6,7,14] that have previously established the importance of the amygdala in conditioned fear using more traditional approaches, such as those based on Pavlovian fear conditioning. More recent evidence has suggested that the BNST, a structure closely connected to the amygdala, may also play an important role in fear or anxiety processes [18,21,22,25]. Our own findings [12] have demonstrated that the infusion of a corticotropin releasing factor (CRF) antagonist, D-Phe CRF, into the BNST disrupts the expression of conditioned defeat. Although these results indicate the involvement of the BNST in the neural circuitry mediating conditioned defeat, a careful examination of the role of this structure as it relates to both the acquisition and expression of conditioned defeat has yet to be conducted. The purpose of the present experiment was to therefore examine the effects of temporary inactivation of the BNST on the acquisition and expression of conditioned defeat.

Methods and Procedures

Animals and housing conditions

Subjects were adult male Syrian hamsters (*Mesocricetus auratus*, Charles River Laboratories, Wilmington, MA) that weighed 110-135 g and were approximately 9-10 weeks old at the time of testing. All subjects were individually housed for 2-3 weeks prior to the start of testing in a temperature ($20 \pm 2^\circ \text{C}$) and humidity-controlled room with free access to food and water. Animals were kept on a 14:10 h light:dark cycle (lights out at 1100 h). All training and testing sessions were performed under dim red illumination during the first 3 hours of the dark phase of the light-dark cycle to minimize circadian effects. Resident aggressors used for defeat training were older (> 6 months), singly housed males weighing between 160 and 180 g. Younger males (2 months) that weighed between 100 and 110 g were group housed (5-6 per cage) and served as non-aggressive intruders. The cages of the experimental animals and the resident aggressors were not changed for 2 weeks prior to testing. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Surgical procedures

Subjects were anesthetized with sodium pentobarbital (90 mg/kg, i.p.) and placed into a stereotaxic frame. Two stainless steel guide cannula (26-gauge) were implanted bilaterally into

the brain and aimed at the bed nucleus of the stria terminalis (1.7mm rostral and $\pm$ 3.6mm lateral relative to bregma). The guide cannula was lowered to 2.1mm below dura to prevent tissue damage to the BNST. On the day of injection, a 33-gauge injection needle that projected 4.2 mm below the guide cannula, reaching a final depth of 6.3 mm below dura, was used for the drug injections. Following surgery, dummy stylets were placed in the guide cannula to help maintain patency. Hamsters were allowed 7-10 days to recover from surgery prior to the start of behavioral testing, and they were handled each day after surgery by gently restraining them and removing and replacing the dummy stylet in order to maintain patency and to habituate the subjects to the infusion procedure.

Social defeat and behavioral testing

On the day of social defeat training, animals were transported to the testing room and allowed to acclimate to the testing room for 1 hour. Training sessions consisted of one 15-min exposure to the RA in the aggressor's home cage, upon which the RA reliably attacked the experimental hamsters within 60 s. This training duration is based on previous studies from our lab [11,12, 16] showing that 15 minutes of defeat resulted in reliable levels of conditioned defeat when subjects were tested with a NAI 24 hours later. Additionally, it should be noted that during a training session, attacks by the RA against a subject occur sporadically, or in bouts, and do not last for the entire duration of the training. All training and testing sessions were recorded on a DVD disc via a CCD camera positioned overhead. Analysis of the initial defeat sessions (see description below) revealed no significant behavioral differences between muscimol and vehicle infused animals, indicating that muscimol infusions did not alter the behavior of the RA's or experimental animals during training (data not shown). Twenty-four hours later, subjects were again transported to the testing room and a NAI was introduced into the subjects' home cage for 5 min. All testing sessions were also recorded on DVD. The trials were scored by an observer that was experimentally blind to the conditions using the behavioral scoring analysis program Hindsight (developed by Scott Weiss, Ph.D.). The total duration of four classes of behaviors were scored during the test session: (a) social behavior (stretch, approach, sniff, nose touching and flank marking), (b) non-social behavior (locomotion, exploration, grooming, nesting, feeding and sleeping), (c) submissive/defensive behaviors (flight, avoidance, tail up, upright, side defense, full submissive posture, stretch attend, head flag, attempted escape from cage) (d) aggressive behaviors (upright and side offense, chase and attack, including bite).

Drug Infusion

Muscimol (Sigma, 1.1 nmol or 2.2 nmol in 200 nL saline) or vehicle control (200 nL saline) was infused bilaterally into the BNST over a 1 min period using a 1 μ L syringe and Hamilton mini-pump connected to a 33 gauge needle via polyethylene tubing. The needle was kept in place for an additional 1-min before being removed to ensure complete diffusion of the drug after which the dummy stylet was replaced. Training or testing began five minutes after infusion.

Site verification

At the end of each experiment, hamsters were administered an overdose of sodium pentobarbital and infused with 200nl of India ink to verify the placement of the injection needle. The brains were then rapidly removed and fixed in 10% buffered formalin for three days before being sectioned on a cryostat. Thirty μ m sections were taken and stained with neutral red and coverslipped with DPX. Sections were then examined under a light-microscope for placement verification. Only animals with injection sites within 0.3mm of the BNST were included in the statistical analysis.

Experiment 1: Role of the Bed Nucleus of the Stria Terminalis in the Acquisition of Conditioned Defeat

The goal of Experiment 1 was to determine whether the temporary inactivation of the BNST using the GABA_A receptor agonist muscimol would block the *acquisition* of conditioned defeat. Animals ($n=40$) were matched by weight and randomly assigned to one of three conditions. Hamsters received one of two doses of muscimol (1.1 or 2.2 nmol in 200 μ L saline) or vehicle control (200 μ L saline) five minutes prior to being placed into the home cage of a resident aggressor for 15 min. On the following day, animals were tested in their own cage against a non-aggressive intruder for 5 min.

In Experiments 1 and 2, we considered the possibility that a difference in drug state between acquisition and expression animals may account for any behavioral differences observed in the subjects. However, previous work from our lab [11] demonstrated that animals with infusions of muscimol both before acquisition and expression still exhibit low levels of submissive/defensive behaviors at levels that were not significantly different compared to those observed in animals receiving muscimol only before defeat or testing. Thus any effect observed in the present study is not likely to be due to a state dependency effect.

Experiment 2: Role of the Bed Nucleus of the Stria Terminalis in the Expression of Conditioned Defeat

The goal of Experiment 2 was to determine whether the temporary inactivation of the BNST using muscimol would block the *expression* of conditioned defeat. Animals ($n=50$) were matched by weight and randomly assigned to one of three conditions and placed into the home cage of the resident aggressor for 15-min. On the following day, animals received one of two doses of muscimol (1.1 nmol or 2.2 nmol in 200 μ L saline) or vehicle control (200 μ L saline) five minutes prior to conditioned defeat testing by placing the non-aggressive intruder into its cage for 5 min.

Statistical Analysis

The total duration (seconds) of each behavior displayed (Submissive/Defensive, Social, Nonsocial) was determined, and the mean total duration of each behavior was then analyzed using a one-way between-subjects analysis of variance (ANOVA) with dose as the between-subjects factor. Statistically significant differences were further analyzed using a Tukey-Kramer multiple comparison post-hoc test to compare all pairwise differences among group means. Significant differences for all analyses were set at $P<0.05$.

Results

Experiment 1: Effect of muscimol in the BNST on Acquisition of Conditioned Defeat

Figure 1a shows the injection sites for animals in Experiment 1. The injection needles were localized primarily within the area of the BNST surrounding the anterior commissure. Only infusion sites within 0.3 mm of the BNST were included in the analysis and 34 of the 40 animals met these criteria. The remaining animals had cannula placements that were localized outside of the BNST, including the caudate nucleus ($n=2$) and the lateral ventricle ($n=1$). There were no differences in submissive behaviors between the group with missed cannula placements and vehicle control animals during testing with the NAI, indicating the site-specific nature of these effects. Infusion sites for 3 animals could not be verified due to occluded cannula at the time of the dye injection, and these animals were removed from the study.

As shown in Figure 2, there was no significant difference between the muscimol infused animals and the saline control groups on any behavioral measure, including duration of submissive/defensive behaviors and social investigation of the NAI ($P>0.05$).

Experiment 2: Effect of muscimol in the BNST on Expression of Conditioned Defeat

Figure 1b shows the injection sites for animals in Experiment 2. Injection needles were again localized primarily within the area of the BNST surrounding the anterior commissure in 43 of the 50 animals, and only those within 0.3 mm of the target site were included in the analysis. Among the missed placements, the cannula was localized entirely within the caudate putamen ($n=3$) or anterior commissure ($n=4$), and these animals showed similar levels of submissive/defensive behaviors compared to controls. These animals were not included in the analysis.

Six hamsters with cannula aimed at the BNST had small amounts of dye in the lateral ventricle following histological analysis. In order to verify that the behavioral effect of BNST injections was not due to leakage into the ventricular system, we added a control study comparing the effects of saline (200 nL; $n=6$) and muscimol (2.2 nmol dissolved in 200 μ L saline; $n=9$) injected directly into the lateral ventricles on the expression of conditioned defeat. Animals were defeated as in Experiment 1 and 2, and, 24 hours later, injected with saline or muscimol immediately prior to testing with a NAI. As shown in Figure 3, there were no significant differences in submissive/defensive behaviors between the two groups, indicating that the small amount of muscimol that diffused into the lateral ventricles did not affect conditioned defeat.

Figure 4 shows the total duration of behaviors exhibited during testing with the NAI following infusions of muscimol or saline. Results indicate that compared to the saline group, animals infused with either dose of muscimol (1.1 and 2.2 nmol) exhibited significantly lower levels of submissive/defensive behaviors ($P<0.001$) and higher levels of non-social behaviors ($P<0.001$). Non-social behaviors consisted of locomotor activity, rearing and digging into the bedding material. There was no evidence of stereotyped behaviors or ataxia as a result of muscimol infusions, suggesting that the decrease in submissive/defensive behavior was not due simply to a competing, non-specific effect of muscimol on behavior.

Discussion

This experiment demonstrates that the infusion of the GABA_A agonist muscimol into the BNST blocks the expression but not acquisition of conditioned defeat in Syrian hamsters. Animals that were infused with muscimol prior to initial defeat training (Experiment 1) did not significantly differ in the duration of submissive or other behaviors compared with vehicle control animals during testing with the NAI 24 hours later. In contrast, the administration of muscimol into previously defeated animals just prior to testing with the NAI (Experiment 2) significantly reduced submissive behaviors compared to control animals. Together, these findings indicate that the BNST may be critically involved in the expression, but not acquisition, of conditioned defeat and suggest that there is an important differentiation in the roles of the lateral BNST and its closely associated amygdalar nuclei.

One possible alternative explanation for the present findings is that the infusion of muscimol may have somehow altered the normal motor response pattern of the subject in a non-specific manner thereby indirectly affecting the expression of submissive behaviors. However, behavioral analysis after drug infusion in both Experiments 1 and 2 showed that muscimol did not induce ataxia or stereotypic behaviors such as circling or digging. Furthermore, an analysis of the training (defeat) sessions revealed no significant differences in the latency to first attack or in overall aggressiveness (counted as total number of attacks) of the RA toward muscimol and saline infused subjects (data not shown), reducing the likelihood that muscimol somehow altered the normal attack pattern of the RA. Additionally, an optimal dose of muscimol was selected based on our previous work that has been shown to interfere with the behavioral expression of conditioned defeat without inducing ataxia or stereotypy [16].

The failure of muscimol to block the acquisition of conditioned defeat in Experiment 1 is consistent with several other studies using a variety of (non-ethologically based) models. For example, LeDoux and colleagues [13] demonstrated that excitotoxic lesions of the BNST failed to disrupt the freezing response that had previously been paired with an acoustic conditioned stimulus. Additionally, using another Pavlovian-based model (fear potentiated startle) Gewirtz and colleagues [9] demonstrated that pre-training lesions of the BNST failed to block the startle response in rats, although lesions of the amygdala significantly disrupted the startle response [24]. Finally, Treit and colleagues [22] also demonstrated the functional specificity of the BNST and amygdala by showing that lesions of the amygdala, but not the BNST, disrupted passive avoidance to an electrified shock probe. These studies, together with the current findings, support the idea that the BNST may not play a key role in the neural circuit modulating the acquisition of fear-based learning, and show for the first time that this lack of involvement may also generalize to more ethologically-based models of conditioned fear.

The results from Experiment 2 suggest that the BNST does play a role in the behavioral expression of conditioned defeat. These findings are consistent with data demonstrating the involvement of the BNST in the expression of other fear-related behaviors. For example, lesions or temporary inactivation of the BNST (but not of the central or basolateral amygdala) impair the behavioral response (freezing and risk assessment) of rats to predator odors [8,15], indicating the involvement of the BNST in the behavioral expression of unconditioned fear. Our lab has also demonstrated that pre-test infusions of the corticotrophin releasing factor (CRF) receptor antagonist, D-Phe into the BNST, but not the amygdala, block the expression of conditioned defeat [12]. These studies, together with the findings from the present study, provide further evidence for a functional dissociation between the amygdala and BNST in the modulation of fear-related behaviors.

Walker and Davis [23] first suggested the possibility of a functional dissociation between the amygdala and BNST and proposed that the BNST is a critical component of a neural circuit modulating ‘sustained’ fear responses (similar to ‘anxiety’) while the amygdala is involved in mediating responses to ‘phasic’ fear response [26]. Several lines of research have supported the double dissociation hypothesis. For example, infusions of the AMPA receptor antagonist NBQX into the BNST, *but not the amygdala*, significantly disrupted startle amplitude in the light-enhanced startle test, a model associated with anxiety, while the infusion of NBQX in the amygdala, *but not the BNST*, significantly disrupted startle amplitude in the fear-potentiated startle model, a test that has been described as more associated with fear [23,24]. In addition, in the predator odor study mentioned above [15], lesions of the BNST specifically blocked the unconditioned freezing response of rats exposed to cat odor, but the same treatment failed to disrupt the defensive response in animals that were confronted with a more salient stimuli (a live cat). In contrast, lesions of the amygdala have been shown to effectively block the defensive behavior in subjects exposed to the live cat [1].

In the context of the present study, defeated animals infused with muscimol prior to testing showed a significant impairment of submissive and defensive behaviors when exposed to the NAI, but muscimol infusions in the BNST prior to the initial defeat session failed to impact submissive behaviors during the testing phase. In contrast, previous studies from our lab have demonstrated that the temporary inactivation of subnuclei of the amygdala (including the basolateral, medial or central nuclei) significantly disrupts the acquisition *and* expression of submissive behaviors [11,16]. One possible explanation of the current findings is that the distinct stimulus properties of the RA vs. NAI stimulate the neural circuit differentially. In particular, the unpredictability of the NAI may require a more sustained (vs. a phasic) response by the subject. Indeed, although the NAI will not attack or otherwise aggressively engage the experimental subject, the behavioral unpredictability of the NAI may instead induce a more diffuse state of sustained defensiveness in the subject that may be more akin to anxiety than

fear. In contrast, the behavior of the subject during the initial defeat session requires a phasic response that is expressed during each (brief) attack by the RA, making the RA a more clearly fearful stimulus. Studies will be conducted in order to further investigate this possibility.

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References

1. Blanchard DC, Blanchard RJ. Innate and conditioned reactions to threat in rats with amygdala lesions. *J Comp Physiol Psychol* 1972;81:281–90.
2. Blanchard DC, Sakai RR, McEwen B, Weiss SM, Blanchard RJ. Subordination stress: behavioral, brain, and neuroendocrine correlates. *Behav Brain Res* 1993;58:113–21. [PubMed: 8136039]
3. Blanchard DC, Spencer RL, Weiss SM, Blanchard RJ, McEwen B, Sakai RR. Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. *Psychoneuroendocrinology* 1995;20:117–34. [PubMed: 7899533]
4. Blanchard RJ, McKittrick CR, Blanchard DC. Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiol Behav* 2001;73:261–71. [PubMed: 11438351]
5. Buwalda B, Kole MH, Veenema AH, Huininga M, de Boer SF, Korte SM, Koolhaas JM. Long-term effects of social stress on brain and behavior: a focus on hippocampal functioning. *Neurosci Biobehav Rev* 2005;29:83–97. [PubMed: 15652257]
6. Campeau S, Miserendino MJ, Davis M. Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. *Behav Neurosci* 1992;106:569–74. [PubMed: 1352104]
7. Cousens G, Otto T. Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behav Neurosci* 1998;112:1092–103. [PubMed: 9829787]
8. Fendt M, Endres T, Apfelbach R. Temporary inactivation of the bed nucleus of the stria terminalis but not of the amygdala blocks freezing induced by trimethylthiazoline, a component of fox feces. *J Neurosci* 2003;23:23–8. [PubMed: 12514197]
9. Gewirtz JC, McNish KA, Davis M. Lesions of the bed nucleus of the stria terminalis block sensitization of the acoustic startle reflex produced by repeated stress, but not fear-potentiated startle. *Prog Neuropsychopharmacol Biol Psychiatry* 1998;22:625–48. [PubMed: 9682277]
10. Huhman KL, Moore TO, Ferris CF, Mougey EH, Meyerhoff JL. Acute and repeated exposure to social conflict in male golden hamsters: increases in plasma POMC-peptides and cortisol and decreases in plasma testosterone. *Horm Behav* 1991;25:206–16. [PubMed: 1648544]
11. Jasnow AM, Huhman KL. Activation of GABA(A) receptors in the amygdala blocks the acquisition and expression of conditioned defeat in Syrian hamsters. *Brain Res* 2001;920:142–50. [PubMed: 11716820]
12. Jasnow AM, Davis M, Huhman KL. Involvement of central amygdalar and bed nucleus of the stria terminalis corticotropin-releasing factor in behavioral responses to social defeat. *Behav Neurosci* 2004;118:1052–61. [PubMed: 15506887]
13. LeDoux JE, Iwata J, Cicchetti P, Reis DJ. Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *J Neurosci* 1988;8:2517–29. [PubMed: 2854842]
14. LeDoux JE. Emotion circuits in the brain. *Annu Rev Neurosci* 2000;23:155–84. [PubMed: 10845062]
15. Markham, CM.; Pentkowski, NP.; Blanchard, DC.; Blanchard, RJ. Abstract Viewer Itinerary Planner. Program No. 784.786. Society for Neuroscience; Washington, D.C.: 2004. Lesions of the anterior and posterior bed nucleus of the stria terminalis differentially affects predatory-stimuli elicited defensive behavior.
16. Markham CM, Huhman KL. Is the medial amygdala part of the neural circuit modulating conditioned defeat in Syrian hamsters? *Learn Mem* 2008;15:6–12. [PubMed: 18174368]

17. McKittrick CR, Magarinos AM, Blanchard DC, Blanchard RJ, McEwen BS, Sakai RR. Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin transporter sites. *Synapse* 2000;36:85–94. [PubMed: 10767055]
18. Meloni EG, Jackson A, Gerety LP, Cohen BM, Carlezon WA Jr. Role of the bed nucleus of the stria terminalis (BST) in the expression of conditioned fear. *Ann N Y Acad Sci* 2006;1071:538–41. [PubMed: 16891614]
19. Potegal M, Huhman K, Moore T, Meyerhoff J. Conditioned defeat in the Syrian golden hamster (*Mesocricetus auratus*). *Behav Neural Biol* 1993;60:93–102.
20. Schuurman T. Hormonal correlates of agonistic behavior in adult male rats. *Prog Brain Res* 1980;53:415–20. [PubMed: 7192876]
21. Sullivan GM, Apergis J, Bush DE, Johnson LR, Hou M, LeDoux JE. 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. *Neuroscience* 2004;128:7–14. [PubMed: 15450349]
22. Treit D, Aujla H, Menard J. Does the bed nucleus of the stria terminalis mediate fear behaviors? *Behav Neurosci* 1998;112:379–86. [PubMed: 9588484]
23. Walker DL, Davis M. Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J Neurosci* 1997;17:9375–83. [PubMed: 9364083]
24. Walker DL, Davis M. The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. *Pharmacol Biochem Behav* 2002;71:379–92. [PubMed: 11830172]
25. Walker DL, Toufexis DJ, Davis M. Role of the bed nucleus of the stria terminalis versus amygdala in fear, stress, and anxiety. *Eur J Pharmacol* 2003;463:199–216. [PubMed: 12600711]
26. Walker DL, Davis M. Role of the extended amygdala in short-duration versus sustained fear: a tribute to Dr. Lennart Heimer. *Brain Struct Funct* 2008;213:29–42. [PubMed: 18528706]

Figure 1.

1a and 1b. Histological reconstructions of injection sites for animals receiving infusions of muscimol into the BNST in Experiments 1 (a) and 2 (b). The shaded area encompasses the injection sites for all animals with cannulas localized in the BNST, and squares represent misplaced injection sites. Drawings are adapted from Morin and Wood (2001).

Figure 2.

Total duration (mean $\pm$ S.E.M.) of behaviors exhibited by defeated animals during the 5-min test with a non-aggressive intruder (NAI). Animals received bilateral infusions of muscimol or saline into the BNST immediately prior to being defeated for 15 min.

Figure 3.

Total duration (mean $\pm$ S.E.M.) of behaviors exhibited by defeated animals during the 5-min test with a non-aggressive intruder (NAI). Animals received bilateral infusions of muscimol or saline into the lateral ventricle immediately prior to testing with the NAI.

Figure 4.

Total duration (mean $\pm$ S.E.M.) of behaviors exhibited by defeated animals during the 5-min test with a non-aggressive intruder (NAI). Animals received bilateral infusions of muscimol or saline into the BNST immediately prior to testing with the NAI. *indicates significant differences compared with saline controls at $p < .0001$.