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Lesions of the basolateral amygdala reverse the long-lasting interference with shuttle box escape produced by uncontrollable stress

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Abstract

Exposure to an uncontrollable, but not a controllable, stressor produces a constellation of behaviors called learned helplessness. In rodents, uncontrollable stress interferes with the ability to learn to escape from escapable shocks delivered in a shuttle box. The stress-induced shuttle box escape deficit is a common screening tool for potential antidepressant strategies. Inconsistencies in the literature exist regarding the time-course of, and mechanisms underlying, stress-induced escape deficits. When no common cues are shared between the stressor and testing environment, the escape deficit is short lived and independent of conditioned freezing. In contrast, when stress and testing occur in the same or similar environments, the escape deficit is very long-lasting. The current studies address the hypothesis that the long-lived escape deficit produced by uncontrollable stress is dependent upon conditioned fear and the basolateral amygdala (BLA). Rats received bilateral excitotoxic lesions of the BLA 2 wk following uncontrollable foot shocks. One wk after surgery, rats were tested for conditioned freezing and escape behavior in the same shuttle boxes in which prior foot shocks were delivered. Stressed rats with sham lesions displayed robust conditioned freezing and failed to escape during shuttle box testing. Lesions of the BLA eliminated conditioned freezing and completely restored stressed rats' ability to perform the escape contingency. These data indicate that the long-lived stress-induced escape deficit produced under conditions in which the stressor and testing environments share common cues is dependent upon conditioned freezing elicited by the BLA. Results have important implications for the mechanisms underlying learned helplessness phenomena.

Keywords

Learned helplessness; conditioned fear; stress; depression; freezing; inescapable shock; anxiety

Introduction

Exposure to an uncontrollable or inescapable stressor produces a constellation of behavioral consequences that do not occur if the stressor is controllable. The sequela of stress-induced

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behaviors that are sensitive to behavioral control of the stressor have been called learned helplessness [24]. One consequence of uncontrollable, but not controllable, stressor exposure is difficulty learning an instrumental escape task, such as a shuttle box escape task during which rats are required to acquire the escape contingency in order to terminate foot shock [21]. The uncontrollable stress-induced shuttle box escape deficit is often used as an animal model of depression- and anxiety-like behavior and as a screening tool for potential antidepressant compounds [2,18,26,36].

Fear conditioning is an associative learning phenomenon during which a previously salient cue (the conditioned stimulus) is paired with a noxious cue (the unconditioned stimulus). Later, exposure to the conditioned stimulus elicits a fear response even when the unconditioned stimulus is absent. Behavioral freezing is a species-typical conditioned fear response in rats [5]. The expression of conditioned freezing can inhibit motor responses (such as escape behavior) elicited by electrical stimulation of the periaqueductal gray [17] or shock [3,6,7]. It is, therefore, possible that conditioned fear processes could contribute to the escape deficit produced by uncontrollable stress. One argument is that if the stressor and behavioral testing environments used in a learned helplessness experiment share common cues, the resulting escape deficit could be due to the expression of fear conditioned to those cues during stressor exposure [19,31]. Additionally, even if cues are not identical between the stressor and testing environments, fear acquired during stressor exposure could be generalized, or transferred, to cues present in the behavioral testing environment that even vaguely resemble those present during stress. Finally, stressor exposure can enhance later fear conditioning [1,33], such as to the shuttle box environment during behavioral testing [19,22]. This enhanced level of fear during behavioral testing could also interfere with shuttle box escape performance.

The potential role of conditioned fear in short-term escape deficits produced by uncontrollable stress was addressed by Maier and colleagues. In a series of studies, this group demonstrated that shuttle box escape deficits produced by uncontrollable stress can be independent of fear. First, little to no freezing is observable in the shuttle box testing environment following exposure to uncontrollable tail shocks delivered in restraint tubes [11,12,19], indicating that minimal fear transfers from the stressor to the testing environment under these conditions. Second, both conditioned fear memory [9] and stress-induced sensitization of later fear learning [34] are very long lasting. When stressor exposure and escape testing occur in different environments, however, the escape deficit is short-lived – persisting only about 48 hr [20]. Third, 10 shocks administered in one environment are more than sufficient for the acquisition of fear conditioning to that environment and later sensitization of new fear learning [34], but are insufficient to interfere with shuttle box escape tested in a different environment [40]. Many more shocks are required. Finally and most importantly, although administration of benzodiazepines immediately prior to stress can prevent the development of the escape deficit [4], manipulations that remove fear during shuttle box escape testing, such as benzodiazepines [4,19] or electrolytic lesions of the amygdala [22], do not prevent shuttle box escape failures 24 hr following uncontrollable tail shocks administered in restraint tubes. These data indicate that, under conditions in which there is no transfer of fear between the stressor and testing environments, stress-induced escape deficits are clearly independent of fear. Maier and colleagues have demonstrated that these short-lived shuttle box escape deficits produced by uncontrollable stress depend instead on transient sensitization of serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN; [27]). Pre-stress benzodiazepines therefore likely prevent the development of the stress-induced escape deficit by inhibiting activity of DRN 5-HT neurons and preventing their sensitization [4,26,41], rather than by reducing fear.

Although escape deficits produced by uncontrollable stress can be independent of fear, a role for fear in uncontrollable stress-induced escape deficits under experimental conditions in which fear is conditioned or transferred to the shuttle box testing environment remains a possibility.

This is an important issue, because the experimental conditions required to produce fear-independent escape deficits are likely very specific and not often met in the laboratory. Indeed, experimenters must be particularly attentive to avoid accidentally introducing common cues to the stressor and testing environments. Moreover, examination of the literature reveals that uncontrollable stress is often delivered through foot shocks in chambers which are either the same as, or very similar to, the shuttle box used for later escape testing ([2,8,13-15,²⁸,35,37,42,43] among many others). Under these conditions, several contextual or discrete cues, such as box shape, grid floor, or foot shocks themselves, at minimum, are frequently common to both the stressor and testing environments. Consistent with a role for fear, when stress and escape testing occur in similar environments, the escape deficit persists much longer than 48 hr [2,10,13,37,38,43]. In fact, we have observed that rats exposed to uncontrollable shocks (delivered to either the tail or feet; [10]) while confined to one side of a shuttle box display an inability to escape from escapable shocks delivered in the same shuttle box up to at least 6 wk later [13]. The long-lasting escape deficit that can be observed when the stressor and testing environments share common cues is independent of 5-HT sensitization [27,39]. Independent mechanisms, therefore, seem to underlie stress-induced escape deficits depending on the relationship between the stressor and testing environments. Whereas 5-HT sensitization can explain short-lived stress-induced escape deficits observed in testing environments very different from the stressor environment, the expression of conditioned freezing could explain the persistent interference with escape learning present in environments that share common cues with the original stressor environment.

The goal of the current study was to determine if the long-lived escape deficit produced by uncontrollable stress when the stressor and testing environments share common cues is dependent upon the expression of conditioned fear during behavioral testing. The approach was to remove fear conditioned to the shuttle box with post-stress lesions of the basolateral amygdala (BLA), which is necessary for the expression of conditioned freezing [30]. We hypothesized that the removal of fear conditioned to the stressor environment with BLA lesions would restore rats' ability to learn to escape from escapable shocks delivered in that same environment weeks later. Results indicate that the long-lived escape deficit produced by uncontrollable stress is dependent upon conditioned fear and help clarify inconsistencies in the literature regarding the time course of, and mechanisms underlying, stress-induced shuttle box escape deficits.

Materials and Methods

Animals

Adult, male Fischer-344 rats weighing 226 ± 3.2 g at the beginning of the experiments were housed in a temperature (22°C) and humidity-controlled environment and were maintained on a 12:12 hr light/dark cycle (lights on 0600-1800). All animals were individually housed in Nalgene Plexiglas cages ($45 \times 25.2 \times 14.7$ cm). Animals acclimatized to these housing conditions and were handled for 1 week prior to any experimental manipulation. Care was taken to minimize animal discomfort during all procedures. All rats were weighed weekly and had *ad libitum* access to food and water. Experimental protocols were approved by the University of Colorado Animal Care and Use Committee.

Shock administration

Uncontrollable foot shocks were administered in the same shuttle box in which later behavioral testing would occur. The entire foot shock procedure lasted 1 hour and occurred in a dark room. Rats were placed into one side of a brightly lit shuttle box (20"W $\times$ 10"D $\times$ 12"H; Coulbourn Instruments, Allentown, PA) with the door separating the two halves of the shuttle box closed. After a 5 minute habituation period, rats received 60 foot shocks (0.6 mA) through the grid

floor. Foot shock duration varied from 5 to 25 sec and occurred on a 1 min variable-interval schedule. This foot shock procedure has been used previously to induce long-lasting interference with shuttle box escape performance [2,13,28]. Foot shocks were delivered during the rats' inactive (light) cycle, between 0800 and 1200. Non-stressed control rats remained in their home cages.

Behavioral Testing

Rats were placed into a brightly lit shuttle box (20"W × 10"D × 12"H; Coulbourn Instruments, Allentown, PA) with the door separating the two halves of the shuttle box open and the room lights extinguished. Rats that received prior foot shock were placed into the same shuttle box in which prior shocks were administered. For the first 5 - 6 min following placement in the shuttle box, freezing was scored using a sampling procedure whereby each subject's freezing behavior was scored every 8 seconds as either freezing or not. Freezing was defined as the absence of all movement except that required for respiration. Following this initial pre-FR-1 freezing observation period, rats received a single fixed ratio-1 (FR-1) trial which was terminated when the rat fully crossed once through the shuttle box door. The latency to escape was recorded and is used as a measure of the rats' ability to simply go through the shuttle box door. The FR-1 trial was followed by another 5 min freezing observation period (post-FR-1 or shock-elicited freezing) to assess the effect of the BLA lesion on shock-elicited freezing. Rats then received 25, 0.6 mA foot shocks that could be terminated by crossing fully to the other side of the shuttle box twice within a 30 s time period (FR-2). FR-2 shocks occurred with an average inter-trial interval of 60 sec and each shock was terminated after 30 sec if an escape response did not occur. A single test session lasted approximately 45 min and was performed by an observer blind to the treatment condition of the animals.

Surgery

Two weeks after uncontrollable shocks in the shuttle box, a total of 41 rats were anesthetized with a combination of ketamine (75 mg/kg i.p.) and medetomidine (0.5 mg/kg i.p.) and mounted into a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). After adjusting head position to place bregma and lambda in the same horizontal plane, small holes were drilled through the skull above the BLA. NMDA (20 µg/µl; Sigma, St. Louis, MO) in sterile saline was infused at a rate of 0.1 µl/min through a 25 gauge, blunt-tipped, 1.0 µl Hamilton syringe attached to a microinjector unit (David Kopf Instruments) mounted on the stereotaxic apparatus at 2 sites bilaterally (2.3 mm posterior to bregma, ± 5.0 mm lateral of bregma, 8.0 mm ventral of the skull surface, total volume 0.25 µl; 3.3 mm posterior to bregma, ± 5.0 mm lateral of bregma, 8.5 mm ventral of the skull surface, total volume 0.25 µl). Coordinates are based on the rat brain atlas by Paxinos and Watson [32]. The needle remained in place for 5 minutes following each injection to allow for diffusion of NMDA away from the injection site. Incisions over the skull were closed with stainless steel wound clips. Atipamezole (0.5 mg/kg i.p.) was administered following surgery to reverse the effects of medetomidine and speed recovery. Animals remained on a heating pad for 1 hour after surgery before being returned to their home cages. Sham rats received the identical treatment except that no injections were made. Rats were tested for freezing and escape behaviors in the shuttle box 1 wk after surgery. The BLA was lesioned after stress in this experiment because post-acquisition, but not pre-acquisition, BLA lesions remove freezing to an environment paired with many shocks [30].

Histology

Lesions were verified using immunohistochemistry for the neuron marker NeuN. Following behavioral testing, rats were killed by rapid decapitation. After extraction from the skull, brains were flash frozen in isopentane chilled to -40°C with dry ice and stored at -80°C until later sectioning on a cryostat. Brain sections (10µm) were thaw-mounted onto gelatin coated slides

and stored at -80°C until processing for NeuN. Slide-mounted brain sections were incubated in 4% paraformaldehyde at room temperature for 1 hour prior to rinses in 0.01M phosphate-buffered saline (PBS). Rinses were followed by a 30 min incubation in 0.3% hydrogen peroxide in PBS. Sections were then incubated at room temperature for 1 hour in blocking solution containing 0.5% Triton X-100, 5% normal goat serum, and 0.5% bovine serum albumin in PBS. Following this blocking step, sections were incubated at 4°C for 48 hours in blocking solution containing a 1:10,000 dilution of monoclonal mouse anti-NeuN (Millipore, Billerica, MA). This incubation was followed by another series of washes in PBS after which the sections were incubated at room temperature for 2 hours in blocking solution containing a 1:200 dilution of biotinylated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA). Sections were then incubated with avidin-biotin-horseradish peroxidase complexes (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) in PBS containing 0.5% Triton X-100 for 2 hours. After washes with PB, sections were placed in a solution containing 3,3'-diaminobenzidine (DAB), ammonium chloride, cobalt chloride, nickel ammonium sulfate, and glucose oxidase in PB for 10 minutes. The peroxidase reaction was started by addition of glucose solution and reacted for 15 minutes, yielding a dark brown/black reaction product. The reaction was stopped by rinses in PBS. Slides were dehydrated in graded alcohol, cleared with histoclear, and cover slipped with DPX. Sections were inspected at $40\times$ magnification under a light microscope and locations of lesions were recorded.

Statistical Analysis

Freezing was collapsed into 1 minute blocks and analyzed with repeated measures ANOVA with stress (no stress vs. uncontrollable stress) and lesion (sham vs. lesion) as the factors. FR-2 escape latencies were collapsed into blocks of 5 trials and analyzed with repeated measures ANOVA. The average freezing scores for the entire pre-FR-1 freezing period, and FR-1 and FR-2 escape latencies, were also analyzed with ANOVA. ANOVAs were followed by Fisher protected least significant difference post-hoc analysis when required. Group differences were considered significant when $p < .05$.

Results

The NMDA injection procedure resulted in a complete bilateral lesion of the BLA in 18 out of 22 rats. The extent of the BLA lesion in rats included in the analysis is shown in Figure 1. In some cases lesions extended slightly into the central nucleus of the amygdala and/or the piriform cortex. Exclusion of rats with unilateral or misplaced lesions yielded the following group sizes: *no stress / sham* ($n = 8$); *stress / sham* ($n = 9$); *no stress / lesion* ($n = 9$); *stress / lesion* ($n = 9$). Figure 2 shows photomicrographs of the BLA from representative sham and NMDA lesioned animals.

Effect of BLA lesions on pre-FR-1 fear expression

The effect of sham surgery or BLA lesion on pre-FR-1 freezing behavior across 1 minute freezing blocks is shown in Figure 3A. Rats exposed to prior uncontrollable stress in the shuttle boxes demonstrated robust conditioned freezing when re-exposed to the same shuttle box 3 wk later. Bilateral lesion of the BLA eliminated conditioned freezing to the shuttle box in previously stressed rats. Repeated measures ANOVA revealed reliable main effects of stress ($F(1, 31) = 119.62$; $p < .0001$), lesion ($F(1, 31) = 80.62$; $p < .0001$), and time ($4, 124 = 3.08$; $p < .05$). The interactions between stress and lesion ($F(1, 31) = 72.85$; $p < .0001$) and stress and time ($F(4, 124) = 3.67$; $p < .05$) were also significant. No other interactions reached significance. The *stress / sham* group differed from all other groups during all 5, one minute freezing blocks. The *stress / lesion* group differed from both *no stress* groups during the first 2 freezing blocks. Figure 3B shows the average freezing scores over the entire 5 minute observation period.

Effect of BLA lesions on FR-1 escape performance

The effect of sham surgery or BLA lesion on FR-1 escape latency is shown in Figure 4. Prior stress administered in the same environment as testing interfered with FR-1 escape performance in sham animals only. Lesions of the BLA eliminated the effect of prior stress on FR-1 escape. The main effects of stress ($F(1, 31) = 10.87$; $p < .05$), lesion ($F(1, 31) = 10.32$; $p < .05$), and the interaction between stress and lesion ($F(1, 31) = 9.59$; $p < .05$) all reached significance.

Effect of BLA lesions on shock-elicited freezing

Freezing was again measured for 5 minutes following the FR-1 trial to verify that a reminder shock would not restore high levels of fear in previously stressed, lesioned animals. The FR-1 shock had no effect on freezing behavior of previously stressed, sham operated rats whose freezing remained at a high level throughout both the pre- and post-FR-1 freezing periods (Figure 5). During the minute immediately following the FR-1 trial, freezing behavior of all other groups increased to an average of $40.3 \pm 3.6\%$ and then rapidly returned to low levels. These data are consistent with previous reports that BLA lesions do not remove the freezing that is present immediately following a single shock [29,44]. Importantly, previously stressed rats with BLA lesions demonstrated freezing behavior identical to non-stressed rats, indicating that the reminder shock did not reinstate high levels of fear in stressed, BLA-lesioned animals. Repeated measures ANOVA revealed reliable main effects of stress ($F(1, 31) = 94.02$; $p < .0001$), lesion ($F(1, 31) = 63.23$; $p < .0001$), and time ($F(4, 124) = 13.27$; $p < .0001$) on post FR-1 freezing. The interactions between stress and lesion ($F(1, 31) = 50.97$; $p < .0001$), stress and time ($F(4, 124) = 4.1$; $p < .05$), lesion and time ($F(4, 124) = 2.75$; $p < .05$), and stress, lesion, and time ($F(4, 124) = 6.47$; $p < .0001$) were all significant. During all 5, one minute blocks the *stressed/sham* group differed from all other groups which never differed from each other.

The effect of BLA lesions on FR-2 escape performance

Figure 6A shows the effects of prior stress and BLA lesions on FR-2 escape performance. Prior stress interfered with FR-2 escape in sham animals only. Post-stress BLA lesions restored the ability to escape from FR-2 trials. Repeated measures ANOVA revealed significant main effects of stress ($F(1, 31) = 35.67$; $p < .0001$), lesion ($F(1, 31) = 19.37$; $p < .05$), and trial block ($F(4, 124) = 5.94$; $p < .05$), and a significant interaction between stress and lesion ($F(1, 31) = 11.53$; $p < .05$). The *stress same/sham* group differed from all other groups during all five trial blocks. The *stress same/lesion* group differed from the *no stress/lesion* group during the first trial block. No other group differences were significant. The average escape latencies across all 25 FR-2 trials are shown in Figure 6B.

Discussion

When re-exposed to the stressor environment 3 wk after uncontrollable stress, rats displayed conditioned fear to the stressor environment accompanied by deficits in FR-1 and FR-2 escape performance. This long-lasting interference with shuttle box escape was dependent upon the expression of conditioned fear, because removal of conditioned freezing with bilateral BLA lesions restored previously stressed rats' ability to perform the escape contingency. These data indicate that fear conditioning can contribute to stress-induced escape deficits under conditions in which fear is transferred from the stressor to the behavioral testing environment.

One issue is how BLA lesions prevent the expression of conditioned freezing. Because rats with BLA lesions can still express shock-elicited freezing ([29,44] and Figure 5), BLA lesions do not remove the ability to freeze. Instead, the current data are consistent with prior observations suggesting that the BLA supports long-term conditioned fear memory [9,30]. It

is therefore possible that stressed rats with BLA lesions learn to escape because they no longer remember the traumatic experience. Regardless of whether BLA lesions prevent the ability to freeze or disrupt fear memory, BLA lesions clearly eliminated conditioned freezing and restored stressed rats' ability to escape. Interestingly, these data are similar to the observation that war veterans with combat-induced damage to the anterior temporal lobe (including the amygdala) are less likely to suffer from post-traumatic stress disorder [16].

The expression of conditioned fear could interfere with shuttle box escape performance by inhibiting motor activity (performance) or by interfering with learning the instrumental escape contingency. The current data support the first of these possibilities. If the escape deficit was caused by a learning failure as opposed to a performance deficit, stressed rats would be expected to be able to rapidly escape FR-1 shocks; an act simply requiring an activity burst most likely representing an unconditioned response to shock [6]. Rats exposed to uncontrollable stress in the shuttle boxes, however, demonstrated a deficit in FR-1 escape. The observed deficit in FR-1 escape suggests that stressed rats' inability to perform the FR-2 escape contingency was caused by a performance deficit. Interestingly, the effect of prior stress on FR-1 escape observed under the current experimental conditions is very different from what is typically observed when stress and testing occur in unique environments. When the stressor and shuttle box testing environments are sufficiently different to prevent the transfer of fear, prior stress does not interfere with the first FR-1 escape trial, even when testing occurs only 24 hr following stress [11,12,19]. It is not surprising that the performance of an escape response is incompatible with conditioned freezing. Fanselow (1991) has suggested that animal defensive responses are hierarchically organized. According to this view, freezing is the primary response to innate or learned aversive stimuli and can inhibit active forms of defensive behavior [7]. Indeed, the presence of contextually conditioned fear is sufficient to inhibit the running and jumping activity burst elicited by either foot shock [6] or electrical stimulation of the dorsal lateral periaqueductal gray [17]. This does not imply that stress does not interfere with later instrumental learning. In the case of shuttle escape learning under the current experimental conditions, rats expressing fear simply do not have the opportunity to learn the escape contingency because they are freezing and freezing precludes moving through the shuttle box door. The current results extend prior work in this area by indicating that fear-elicited inhibition of active defensive behavior is dependent on the BLA.

Based on the current results, we can conclude that there are 2 distinct types of shuttle box escape deficits produced by uncontrollable stress which differ in their time courses and mechanisms of expression. Under experimental conditions which ensure minimal transfer of fear from the stressor environment to the testing environment, the uncontrollable stress-induced escape deficit lasts only about 48 hr [20], is dependent upon 5-HT sensitization [23,27], and is independent of conditioned fear [19,22]. This escape deficit could represent a true stress-induced impairment of instrumental learning. On the other hand, under experimental conditions in which fear is conditioned or transferred to the escape testing environment, for example when the stressor and testing environments share common cues, the resulting escape deficit is long lasting, is independent of 5-HT sensitization [27,39], and is dependent upon conditioned fear and the BLA (Figure 6).

Although there are clearly multiple ways in which to produce an escape deficit, escape deficits of any kind have traditionally been referred to as "learned helplessness." The allusion to all escape deficits (including fear-independent and fear-dependent stress-induced escape deficits) in the literature as learned helplessness has resulted in confusion over the time course and underlying mechanisms of stress-induced shuttle box escape deficits. Bearing in mind that blockade or reversal of stress-induced shuttle box escape deficits is a common screening tool for potential antidepressant or anxiolytic compounds, the implications of this confusion are obvious. For example, a compound that reverses the long-lasting, fear-dependent escape deficit

might have done so only because it reduces the expression of conditioned freezing and not because it is an effective antidepressant. Moreover, stating that this compound reverses learned helplessness, although a common practice; would be misleading. The long-lasting stress-induced escape deficit dependent upon fear conditioning is not, by definition [25], learned helplessness (for further discussion of this issue, see [27] and [10]). This does not imply that one method of producing an escape deficit is a better animal model than another, only that researchers should be aware that the different types of stress-induced escape deficits necessitate distinct interpretations regarding underlying mechanisms depending on the methods employed. The current data help clarify inconsistencies in the literature regarding features of the stress-induced escape deficit, and illustrate the importance of standardizing terminology in animal behavioral experiments.

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Figure 1.

Schematic representation of the extent of damage produced by NMDA infused into the region of the basolateral amygdala for the animal showing the minimum amount of damage (dark gray) and the animal showing the maximum amount of damage (light gray). Values represent distance from bregma. Coronal brain section images adapted from Paxinos and Watson (1998).

Figure 2. Representative coronal brain slices showing the extent of the NMDA lesion of the basolateral amygdala. The box outline in (A) shows the region magnified in the photomicrographs from a sham animal (B) and a lesioned animal (C) stained with NeuN.

Figure 3.

Freezing behavior of rats following sham surgery or post-stress lesions of the basolateral amygdala (BLA). One week following no shocks (No Stress) or uncontrollable shocks in the shuttle box (Stress), rats either received sham surgery (Sham) or NMDA infusion bilaterally into the BLA. Two weeks later, rats were re-exposed to the shuttle box for assessment of freezing behavior. Data are expressed as A) 1-min freezing blocks or B) the average freezing score across the entire observation period. Data represent group means $\pm$ SEM. * $p \leq .05$ relative to all other groups.

Figure 4.

Fixed ratio-1 (FR-1) escape performance of rats following sham surgery or post-stress lesions of the basolateral amygdala (BLA). One week following no shocks (No Stress) or uncontrollable shocks in the shuttle box (Stress), rats received either sham surgery (Sham) or NMDA infusion bilaterally into the BLA. Two weeks later, rats were re-exposed to the shuttle box and FR-1 escape latency was recorded. Data represent group means $\pm$ SEM. * $p \leq .05$ relative to all other groups.

Figure 5.

Shock-elicited (post-fixed ratio-1; FR-1) freezing behavior of rats following sham surgery or post-stress lesions of the basolateral amygdala (BLA). One week following no shocks (No Stress) or uncontrollable shocks in the shuttle box (Stress), rats received either sham surgery (Sham) or NMDA infusion bilaterally into the BLA. Two weeks later, rats were re-exposed to the shuttle box and shock-elicited freezing was scored immediately following a single FR-1 trial. Data represent group means $\pm$ SEM.

Figure 6.

Fixed ratio-2 (FR-2) escape performance of rats following sham surgery or post-stress lesions of the basolateral amygdala (BLA). One week following no shocks (No Stress) or uncontrollable shocks in the shuttle box (Stress), rats received either sham surgery (Sham) or NMDA infusion bilaterally into the BLA. Two weeks later, rats were re-exposed to the shuttle box and FR-2 escape latency was recorded. Data are expressed as A) blocks of 5 FR-2 escape trials and B) the average escape latency across all 25 FR-2 trials. Data represent group means $\pm$ SEM. * $p \leq .05$ relative to all other groups.