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Short communication

# Excitotoxic lesions of the bed nucleus of the stria terminalis (BNST) attenuate the effects of repeated stress on weight gain: Evidence for the recruitment of BNST activity by repeated, but not acute, stress

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ABSTRACT

Exposure to repeated stress can lead to diverse and widespread behavioral consequences, including reduction in food and water intake and subsequent diminution in weight gain. Many reports have suggested that repeated stress substantially alters the neurochemistry, morphology and physiology of neurons within the bed nucleus of the stria terminalis (BNST). Here we investigate the role of the BNST in mediating the reduced weight gain observed during repeated stress. Rats exposed to a one-week variate stress paradigm exhibited a reduction in weight gain over the course of the 7-day paradigm. Excitotoxic lesions to a subregion of the anterolateral BNST containing the oval nucleus had no effects early in the 7-day paradigm, but significantly attenuated the effects of repeated stress on weight gain by the last day of stress. These data suggest that at least two mechanisms mediate the effects of stress on body weight gain, and that when stressor exposure becomes repeated, the BNST is recruited, worsening the symptoms of stressor exposure.



## 1. Introduction

Repeated exposure to daily stressors produces a variety of behavioral changes that include anorexia and diminution in weight gain in rats [1,2]. Attenuated weight gain in rats is a commonly observed effect of stressor exposure that suggests that the central processing of stressful stimuli may influence feeding behavior and/or metabolic processes; hence, weight changes represent a useful mechanism to assess stress effects and may also reflect processes by which stressor exposure may decrease and/or increase feeding behavior and/or metabolism in other species, including humans. Many behavioral effects of repeated stress have been linked to the activity of corticotropin-releasing hormone (CRH), which is found in high concentrations in the paraventricular nucleus of the hypothalamus (PVN), central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis (BNST). CRH has been well studied for its role in initiating peripheral stress-responses as well as coordinating behavioral responses to stressor exposure. Repeated central CRH injections mimic many stress-induced behavioral changes and can produce anorexia and weight loss [3,4]; by contrast, blockade of CRH receptors during repeated stress or CRH can attenuate these responses [5,6]. Hence, it is likely that the anorexigenic effects of repeated stress are mediated by CRH-expressing neurons in the PVN, CeA, and/or BNST.

BNST neurons respond to a variety of stressful stimuli, and have been implicated in mediating anxiety-like behavior (see Refs. [7,8] for review) and anorexia [9]. In particular, BNST CRH activity has been shown to be critical for the development of many anxiety-like behavioral phenotypes (for review see Refs. [10,11]), and activation of BNST CRH receptors produces an anxiogenic [12] and anorexic state [9]. The same regions of the BNST have been argued to mediate peripheral stress responding via projections to the PVN [13–15]. Moreover, increases in BNST neuroplasticity have been shown to be altered following exposure to repeated stress paradigms [16,17] or repeated administration of drugs of abuse [18,19]. While changes in BNST neuroplasticity have been argued to mediate stress-induced anxiety-like behavioral states, it is still not clear whether BNST activation is necessary for the observation of stress-induced anorexia.

Here we show that a 7-day repeated variate stress paradigm attenuates weight gain throughout the week of stress. Excitotoxic lesions of the BNST during repeated stress attenuated the effects

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of repeated stress on weight gain only towards the end of the 7-day paradigm. These data suggest that when stressor exposure becomes repeated, BNST activity is recruited, enhancing the effects of repeated stress on weight.

## 2. Method

### 2.1. Subjects

Male Sprague-Dawley rats (200–275 g) from Charles River Laboratories (Wilmington, MA) were single-housed with food and water available ad libitum, and kept on a 12 h light/dark cycle (lights on at 7 am). Rats were allowed one week of acclimation upon arrival to the facility prior to experimentation. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Vermont.

### 2.2. Stress procedure

Stressed rats were exposed to a 7-day repeated variate stress paradigm that has been previously described [20]. Briefly, rats were randomly assigned to either stress or control groups. Control rats were weighed daily, but received no other treatment during the 7-day stress period. Stressed rats were weighed daily prior to the administration of one of the following stressors:

#### 2.2.1. Oscillation

Rats were placed inside a plastic chamber 28 cm × 17 cm × 13 cm ($L \times W \times H$), that was secured to a clinical rotator (Fisher Scientific, Morris Plains, NJ), and oscillated at low to medium speed for 30-min.

#### 2.2.2. Forced swim

Rats were placed in a cylindrical container 29 cm × 37 cm ($D \times H$) filled with room temperature water to a depth that prevented the rat tail from touching the bottom. After 5-min of monitored swimming, rats were placed in a holding chamber for 30 min prior to being returned to their home cage.

#### 2.2.3. Footshock

Rats were placed inside a Plexiglas conditioning chamber (Med Associates, St. Albans, VT) 30 cm × 25 cm × 35 cm ($L \times W \times H$). After a 5-min acclimation period, two 1.0 mA 5-s scrambled footshocks were delivered through the grid floor with a 1-min inter-trial interval.

#### 2.2.4. Restraint

Rats were placed in a cylindrical restraining device 9 cm × 15 cm ($D \times H$) for 60-min.

#### 2.2.5. Pedestal

Rats were placed on an elevated platform 20 cm × 20 cm ($L \times W$) that was 60 cm from the floor. Rats remained on the platform for 30-min before being returned their home cages.

Stressed rats received one stressor a day in the following order: oscillation, forced swim, footshock, restraint, pedestal, forced swim, footshock. Rats were returned to their home cages immediately after each stressor.

### 2.3. Drugs

N-methyl-D-aspartate (NMDA) was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in saline 0.9%.

### 2.4. Statistics

A 2 × 2 × 8 (stress treatment × lesion treatment × time) repeated measures analysis of variance (ANOVA) was used to analyze weight data over the entire 7-day stress period using IBM SPSS Statistics Version 19 (International Business Machines, Armonk, NY). Because data did not meet assumptions of sphericity, Greenhouse–Geisser corrections were used. In order to assess effects in the early and late phase of the stress paradigm, one-way ANOVAs and post hoc tests were conducted on the percent weight change from Days 1 to 4, and also on weight change from Days 4 to 8. Because we predicted that the stress–sham group should differ from the other three groups, we followed the one-way ANOVA analyses with a Dunnett's multiple comparison test comparing all groups against the stress–sham rats.

### 2.5. Surgical Procedure

Rats were anesthetized with isoflurane vapor (1.5–3.5%), and secured in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). After incision, the skull was exposed and two drill holes were made at the following coordinates: AP = −.26, ML = ±3.82. For each rat, a 10 μl syringe attached to an infusion pump was stereotaxically placed into the BNST (from bregma in mm, AP = −0.26, DV = −6.8, and ML ± 3.82 at a 20° angle). NMDA was infused (4 μg in 200 nl) into each BNST over 4 min followed by a delay period of 4 min before retracting the syringe to prevent spreading. For sham-operated control animals, an equivolume of saline was infused. After infusion, the wound was closed with wound clips. Once awake, rats were returned to their home cages for 7-day post-surgery recovery, during which all rats were monitored and weighed daily.

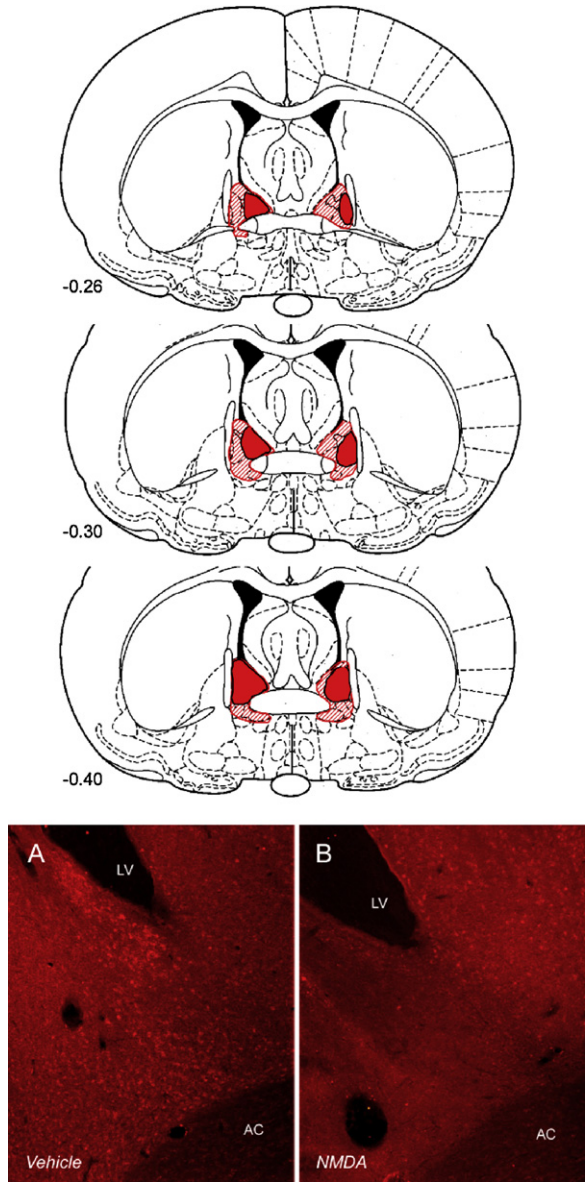


**Fig. 1.** Above: extent of the smallest (solid) and largest (hatched) anterolateral NMDA excitotoxic BNST lesions accepted for analysis. Below: a representative excitotoxic BNST lesion visualized using anti-NeuN immunohistochemistry. BNST tissue from a vehicle treated (sham) rat is shown on the left; the same region is shown from an NMDA (lesion) rat on the right.

After surgery recovery, rats were administered the 7-day repeated variate stress paradigm described above. Twenty-four hours after last stressor exposure (Day 8), rats were weighed and perfused transcardially with saline followed by 4% paraformaldehyde.

### 2.6. Neu-N immunohistochemistry

Excitotoxic lesions were verified using Neu-N immunohistochemistry (see Fig. 1). After perfusion, brains were removed, post-fixed for 48 h at 4 °C in 4% paraformaldehyde, and equilibrated in 30% sucrose and rapidly frozen in a dry-ice slurry for cryosectioning (30 μm). Sections were mounted onto gelatin-coated slides and washed in 0.1 M sodium phosphate buffer. Sections were then permeabilized and blocked in 0.3% triton and 1% bovine serum albumin in phosphate buffered saline for 15 min each, and incubated with mouse monoclonal anti-NeuN antibody overnight at 4 °C. After subsequent washes in buffer, sections