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5-HT_{1B} mRNA expression after chronic social stress

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Abstract

Chronic stress contributes to vulnerability for depression and drug addiction. The function of the serotonergic system has been found to be modified by chronic stress and these changes may play an important role in stress-related relapses to drug craving. The 5-HT_{1B} receptor is expressed in nucleus accumbens projection neurons and modulates drug reward mechanisms and there is evidence suggesting that stress alters the regulation and function of these receptors. To examine the role of these receptors in integrating the effects of stress on reward mechanisms, we examined whether chronic or acute social defeat stress (SDS) regulates 5-HT_{1B} mRNA in dorsal and ventral striatum, regions that are critical for integrating the effects of environmental stresses on reward motivated behavior. In a third experiment, 5-HT_{1B} mRNA regulation in response to another acute stressor, inescapable tailshock, was measured. Our results indicate that intermittent and daily SDS procedures attenuated body weight gain, induced adrenal hypertrophy, and reduced the preference for saccharin, a sweet solution preferred by normal rats. There was a trend for daily, but not intermittent SDS to increase 5-HT_{1B} receptor mRNA levels in nucleus accumbens. Therefore, in the next experiment, we examined daily SDS in greater detail and found that it increased 5-HT_{1B} receptor mRNA expression in rostral nucleus accumbens shell, an area especially associated with reward functions. Neither acute SDS, nor acute tailshock stress had a significant impact on 5-HT_{1B} mRNA expression in the striatum. Since increased 5-HT_{1B} receptor expression in nucleus accumbens shell neurons can facilitate cocaine and alcohol reward mechanisms, this adaptation in endogenous 5-HT_{1B} mRNA may be involved in the SDS-associated increase in vulnerability for developing addiction.

Keywords

chronic stress; serotonin; mesocorticolimbic pathway; 5-HT_{1B} receptor; reward

1. Introduction

Certain types of chronic stress can exacerbate the course of mood disorders [1] and contribute to the vulnerability to initiate drug abuse or trigger relapse [2, 3]. Altered hedonic response to natural rewards is a core feature of both mood disorders and drug addiction, and may be an important interface between stress response and reward mechanisms in the brain

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[4]. The mesocorticolimbic reward pathway is critical in assessing reward (but see [5]), and plasticity in this circuit may contribute to stress-induced anhedonia as well as adaptations related to drug use [4]. Hedonic deficits induced by chronic stress can be detected in the form of decreased preference for a sweet solution [6, 7]; this index of anhedonia can be reversed by antidepressant treatment [8–10].

The mesocorticolimbic circuit for assessing and responding to reward includes the nucleus accumbens (NAc) in the ventral striatum, the dorsal striatum, the ventral tegmental area (VTA) and the medial prefrontal cortex. These regions have also been implicated in certain types of stress, the symptomatology of depression [4], and drug abuse [11]; manipulations of the NAc can produce both pro-depressive and antidepressant effects [4, 12, 13]. Serotonin is a key modulator of stress responses and reward function [14, 15], and several lines of evidence implicate the serotonin-1B (5-HT_{1B}) receptor in NAc neurons as an important mediator of drug and stress adaptations in reward-related behavior. In the NAc shell (NAcSh), 5-HT_{1B} receptors are expressed in medium spiny neurons and are located on axon terminals in targets such as the VTA where they inhibit GABA release [16–18]. These projections to VTA provide inhibitory feedback that reduces subsequent release of dopamine from VTA projections back to NAc [19, 20]; therefore, activation of these 5-HT_{1B} receptors disinhibits dopaminergic function. Available evidence suggests that acute cocaine exposure (both contingent and noncontingent) regulates 5-HT_{1B} mRNA in these neurons, and increased 5-HT_{1B} expression in these neurons enhances a variety of behavioral responses to cocaine, alcohol, and amphetamine [21–24]. There is also evidence that stress regulates these 5-HT_{1B} receptors, but the evidence is less clear [22, 25, 26].

Given the link between hedonic state, reward, stress, and serotonin, it is possible that 5-HT_{1B} receptors in the mesocorticolimbic reward pathway are critical modulators of hedonic state that are regulated by stress and drug experiences and in turn modulate hedonic and drug reward mechanisms. To begin to tease apart the different aspects of these relationships, we have examined the impact of two well-established stress models in rats, social defeat and inescapable tail shock, on 5-HT_{1B} mRNA expression and hedonic behavior. Therefore, we wanted to examine the effect of stress in the absence of cocaine exposure to assess its role in regulating 5-HT_{1B} mRNA expression in the striatum. We used the social defeat model as a chronic and acute stressor, as it is a well-established and reliable method of examining the effects of chronic stress on hedonic and drug addiction mechanisms. For example, it induces anhedonic behavior in the sucrose preference test [7], increases sensitization to psychostimulants, and escalates cocaine self-administration [27, 28]. Additionally, we used acute inescapable tail shock because it has been shown to be a reliable and reproducible method of eliciting stress-induced anhedonia (i.e., decreased consumption of sucrose solution) [29], increases immediate early gene expression in serotonergic neurons [30], and has been shown to facilitate drug – induced dopamine efflux in the NAcSh [31]. Our over-riding hypothesis is that stress exposure increases 5-HT_{1B} mRNA expression in the ventral striatum as a compensatory adaptation to hedonic challenges, but that this increases the rewarding effects of drugs and thereby accelerates the progression of addiction. The hypothesis for the current set of studies is that chronic stress increases expression of 5-HT_{1B} mRNA in the ventral striatum. We will investigate 5-HT_{1B} mRNA regulation along the full extent of the striatum. Our rationale for a rostral-caudal investigation is supported by previous studies that have shown that activating GABA receptors along the rostral-caudal gradient of the NAc elicits behavioral outcomes, in terms of hedonic response [32]. Studies investigating behavioral drug response have also implicated rostral-caudal differences in drug mechanisms [33–35].

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2. Methods and Materials

2.1 Subjects

Male Sprague Dawley rats (250–275g) were two months old at the time of delivery into the animal facility and were used in experiments after a one week acclimation period. Male (375–425g) and female (8 weeks old) Long Evans rats were used as stimulus animals (i.e. resident pairs) only; no dependent measures were collected from these animals. All rats were procured from Harlan Laboratories (Indianapolis, IN). Experimental animals (Sprague Dawley) were individually housed; resident stimulus animals (Long Evans) were pair-housed in a male-female configuration, in a temperature and humidity controlled vivarium, on a 12-12h light-dark cycle, with lights-on at 0600. Food and water were available *ad libitum.* In Experiment 2, food was available *ad libitum* in food hoppers and was weighed daily. All procedures were approved by the Institutional Animal Care and Use Committee. This research was conducted according to the requirements of all applicable local, national, and international standards for the care and use of laboratory animals [36].

2.2 Social Defeat Stress

2.2.1 Residents—Male Long Evans rats were housed for at least four breeding cycles with tubally ligated females (females were tubally ligated to prevent pregnancy while maintaining hormonal tone and sexual receptivity). Males were then screened for aggression [37]. Only those that consistently displayed aggressive behavior toward intruder stimulus animals (which were not used in the following experiments) were included in the study.

2.2.2 Social Defeat—Social defeat procedures were similar to those described by Covington, et. al. [38] and took place between the hours of 0730 and 1100. Briefly, females were removed from residents' cages; an intruder was placed in the resident's cage under a mesh protective enclosure for 10 minutes, resulting in the instigation of aggressive behavior from the resident. The mesh enclosure was then removed, and the animals were allowed to interact. Defeat was characterized by the resident pinning the intruder in a supine position for at least 5 consecutive seconds or the resident biting the intruder five times in a row. Time to defeat averaged between 10 and 60 sec; if there was no defeat by five minutes, the interaction was ended. After defeat, the intruder was placed under the mesh enclosure and remained in the resident's cage for an additional 30 minutes. Intruders were then returned to their home cages and females returned to the respective resident's cage. All animals were monitored for health during the experiment, including assessment of coat condition, body weight, and visual inspection for wounds. Any animal that had a wound that required stitches was removed from the study (two animals met that criterion).

2.3 Saccharin Testing

Animals were presented with two 50mL sipper bottles of liquid for one hour of access between the hours of 1630 and 1800. One bottle contained tap water and the other had 0.1% saccharin in tap water. In Experiment 2, animals had one drink session exposure to establish baseline drinking and one exposure after the last defeat episode to determine the effects of stress on consumption of the sweet liquid. In Experiment 3, animals had two drink session exposures on two consecutive days, and then testing on 4, 7, and 10 days after the onset of stress to determine the time course of stress-induced saccharin drinking deficits. Baseline drinking in Experiment 3 was an average of the two exposure days. The saccharin testing protocol was developed with data from pilot studies that determined the best time of day and length of exposure for detecting stress-induced deficits in intake. Animals that drank less than 2 mL of liquid during the baseline exposures were excluded from the study (approximately 5%).

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2.4 Experimental Design

Figure 1 depicts the experimental designs. In Experiment 1, animals were exposed to either daily social defeat stress (10 days), intermittent (every other day for 10 days; 5 days total) stress, or remained in home cages as controls. Before the onset of stress, these animals were exposed to a 0.1% saccharin solution; their preference for the solution was then tested 8 hours after the last defeat episode (Figure 1A). Experiment 2 was a follow-up to Experiment 1, and was designed to measure NAc 5-HT_{1B} mRNA along the entire rostral-caudal axis. Therefore, in Experiment 2, animals were either socially defeated daily for 10 days or remained in home cages as controls. These groups were exposed to the 0.1% saccharin solution on two separate days before the onset of stress, which reduced baseline variability, and then after 4, 7, and 10 days of stress. Saccharin preference testing took place 8 hours after defeat (Figure 1B) to allow the time to recover from the stress procedure. Since acute exposure to cocaine, a rewarding substance, has been shown to increase 5-HT_{1B} mRNA in the NAcSh, but extended saccharin access does not [26], saccharin testing was restricted to only the chronic stress groups. Additionally, animals in Experiments 1 and 2 were sacrificed 24 hours after the last social defeat, to avoid possible effects of saccharin on 5-HT_{1B} mRNA expression. In Experiment 3, animals were exposed to a single social defeat. They were sacrificed 24 hours later and brains were processed for *in situ* hybridization (Figure 1C). Information on the tailshock stress experiment can be found in the Supplemental methods and materials section.

2.5 Tissue collection

Four hours after the end of the tailshock session or 24 hours after the last social defeat episode, animals were sacrificed via CO_2 inhalation. Brains were extracted and flash frozen in isopentane cooled on dry ice and then stored at −70°C until processing. Tissue was sliced at 20μm on a Leica CM3000 frozen cryostat (Bannockburn, IL) and mounted on Fisherbrand Superfrost glass microscope slides (Pittsburgh, PA). Slides were stored in boxes with desiccator pellets at −70°C until the *in situ* hybridization assay was performed. Adrenal and thymus glands were collected into saline filled 12- or 24-well plates on wet ice (Experiment 2). Organs were cleaned and weighed on the day of processing.

2.7 *in situ* hybridization histochemistry

in situ hybridization was performed as previously described [39, 40]; we probed for corticotrophin releasing hormone (CRH) mRNA in the hypothalamus and 5-HT_{1B} mRNA in striatum. Briefly, tissue was fixed, deaminated, delipidated, and dehydrated. For 5-HT_{1B} mRNA, oligonucleotide probes (Sigma Aldrich, St. Louis, Mo) were labeled with ^{33}P α-dATP (MP Biomedicals, Solon, OH) using terminal deoxytransferase (Fermentas, Glen Burnie, Maryland). For CRH mRNA, riboprobes were transcribed with ^{33}P α-dUTP (Amersham) constituting 65% of total UTP and T7 RNA polymerase. Tissue was hybridized in a humidified chamber overnight at 37°C and then washed in 2X sodium chloride/sodium citrate solution for one hour at 55°C and then one hour at room temperature, dehydrated, and exposed to Phosphor Screens (Perkin Elmer). Phosphor screens were developed 24 hours later with the Cyclone storage phosphor scanner (Perkin Elmer, Meridien, CT). *In situ* hybridization signal was quantified using computer-based densitometry software (MCID Analysis 7.0). Background measures were taken from white matter and subtracted from regional measurements to yield integrated gray levels. Measurements were taken from 2–4 sections per animal at each rostral-caudal level studied, and then averaged.

2.8 Statistics

Food, body weight gain, and saccharin consumption were compared using two factor repeated measures ANOVA. Adrenal and thymus weights were compared using a single

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factor ANOVA. 5-HT_{1B} mRNA levels in Experiment 1 were analyzed with a single factor ANOVA. In addition to a single factor ANOVA to determine differences between the three groups, we made one *a priori* planned comparison in which we decided to compare only control and daily groups for NAcSh 5-HT_{1B} expression with a Student's t-test. Our rationale for this specific comparison was that if intermittent stress had an intermediate effect on 5-HT_{1B} mRNA expression, then it may wash out meaningful differences between the control and daily stress groups. 5-HT_{1B} mRNA levels in Experiments 2 and 3 were analyzed using Student's t-test for each individual coordinate along the rostral-caudal axis. Kruskal Wallis ANOVA on Ranks was used when data violated the standard assumptions of ANOVA. Correlation was assessed with Pearson's Product Moment analysis.

3.Results

3.1 Experiment 1

The purpose of this experiment was to determine the impact of social defeat stress frequency on hedonic state and the expression of 5-HT_{1B} receptors in the mesolimbic reward pathway. Both chronic and intermittent social defeat decreased the rate of body weight gain (Figure 2), consistent with other chronic stress paradigms [42–44]. There was a main effect of group ($F_{2, 208} = 4.46$, $p<0.05$), day ($F_{10, 208} = 332.38$, $p<0.001$), and an interaction between group and day ($F_{20, 208} = 2.75$, $p<0.001$) on body weight gain. *Post hoc* tests revealed no differences between groups on days 1–4, but a significant difference between daily social defeat and controls on days 5–11, and a significant difference between intermittent social defeat and controls that manifested on days 7–11. There were no significant differences between daily and intermittent stress duration on body weight gain.

Food was available *ad libitum* in hoppers located inside the cages and was monitored by weighing hoppers daily. Table 1 lists food intake during social defeat. While there was no main effect of group ($F_{2, 179} = 2.11$, $p=0.15$), there was a main effect of day ($F_{9, 179} = 4.81$, $p<0.001$), as well as a group by day interaction ($F_{18, 179} = 1.72$, $p<0.05$). *Post hoc* tests revealed a significant difference between the control and daily social defeat groups on the first day of defeat ($p=0.001$), and the intermittent group was significantly different than both control and daily defeat groups on day 4 ($p=0.02$ and 0.03, respectively). Table 2 lists organ weights. Neither chronic, nor intermittent stress decreased raw thymus weight ($F_{2, 17} = 0.16$, $p=0.85$) or thymus weight adjusted as a percentage of body weight (adjusted weight; $F_{2, 17} = 0.68$, $p=0.52$). Additionally, there were no significant group differences in raw adrenal weight ($F_{2, 17} = 0.20$, $p=0.82$). Adjusted adrenal weight failed the assumptions of standard ANOVA; a Kruskal-Wallis test on Ranks revealed a significant group effect ($p<0.05$) with *post hoc* analysis showing a significant difference between control and daily groups ($p<0.01$), suggesting adrenal hypertrophy in the daily defeated group.

Both daily and intermittent stress decreased consumption of the saccharin solution, compared to controls (Figure 3). There was a main effect of time ($F_{1, 31} = 5.82$, $p<0.05$) and an interaction between group and time ($F_{2, 31} = 8.39$, $p<0.05$), but no main effect of group ($F_{1, 31} = 2.19$, $p=0.15$). More specifically, in the challenge drinking test, control animals significantly increased saccharin intake over baseline ($p<0.001$), while neither the intermittent, nor the daily stressed groups altered drinking behavior over time. Finally, control animals drank significantly more saccharin during the challenge test than intermittent or daily stress groups (P=0.002 and 0.014, respectively).

Figure 4 shows the results of an *in situ* hybridization for 5-HT_{1B} receptor mRNA in ventral striatum for control, intermittent stress, and daily stress groups. 5-HT_{1B} mRNA signal was measured in NAc, in both the core and shell, at approximately 1.2 to 1.0 mm anterior to bregma. A one way ANOVA revealed no group differences in the NAcSh ($F_{2, 18} = 1.17$,

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$p<0.34$) or NAc core ($F_{2, 17} = 0.44$, $p<0.65$), although there was indication of a trend for increased 5-HT_{1B} mRNA in NAcSh in the animals that received daily social defeat, so we investigated this further in Experiment 3, in which we examined 5-HT_{1B} mRNA expression along the rostral to caudal extent of striatum.

3.2 Experiment 2

The purpose of this study was to determine whether 5-HT_{1B} mRNA throughout the striatum was altered after daily social defeat stress. Figure 5 depicts body weight gain over the course of the experiment. There was a main effect of group ($F_{1, 230} = 6.27$, $p<0.05$) and day ($_{10, 230} = 93.11$, $p<0.001$), and an interaction between group and day ($F_{10, 230}= 7.93$, $p<0.001$). More specifically, defeated animals had attenuated body weight gain on days 6–11 compared to controls, consistent with chronic stress.

To determine the time course of chronic stress effects on hedonic deficits, baseline saccharin consumption was measured (the two trials were averaged) and then saccharin preference was tested 4, 7, and 10 days after the onset of daily social defeat stress (Figure 6). There was a main effect of group ($F_{1, 82} = 4.45$, $p<0.05$) and day ($F_{3, 82} = 11.56$, $p<0.001$), as well as an interaction between group and day ($F_{3, 82} = 3.67$, $p<0.05$). Specifically, socially defeated animals consumed significantly less saccharin solution after 7 ($p<0.05$) and 10 days ($p<0.01$) of daily defeat, compared to baseline measurements. Additionally, within group comparisons revealed that control animals drank significantly more saccharin solution over time, while there were no differences between days in the defeated group.

To determine the extent of rostral to caudal expression changes in 5-HT_{1B} mRNA levels in response to chronic stress, 5-HT_{1B} mRNA was measured in the NAc at four levels: 1.6, 1.2, 1.0, and 0.7 mm anterior to bregma. Student's t-test revealed no significant differences in 5-HT_{1B} mRNA expression in the NAcSh at 0.7 ($p=0.66$), 1.2 ($p=0.18$), or 1.6 ($p=0.08$) mm rostral to bregma (Figure 7A). However, chronic social defeat significantly increased 5-HT_{1B} mRNA in the NAcSh at 1.0 mm rostral to bregma ($p<0.05$). In the NAc core, there were no significant group differences at any level assayed; p-values ranged from 0.14 to 0.97 (Figure 7B). Dorsal striatum was analyzed from the rostral-most tip at 2.7 mm anterior to bregma to −0.26 mm caudal to bregma. There were no group effects on rostral-most striatal 5-HT_{1B} mRNA expression (level 2.7 mm rostral to bregma, data not shown; $p=0.65$). Additionally, there were no differences in 5-HT_{1B} mRNA expression in the dorsomedial striatum at any level (p-values ranged from 0.17 to 0.99; Figure 7C). In the dorsolateral striatum, at the level of −0.26 mm caudal to bregma, daily defeated animals had significantly increased 5-HT_{1B} receptor mRNA ($p=0.04$). There were no significant group effects at any other level (p-values ranged from 0.17 to 0.83; Figure 7D). Figure 7E shows a representative picture of 5-HT_{1B} mRNA hybridization signal from a chronically defeated animal. Striatal regions that were quantitated have been circled and include dorsolateral striatum, dorsomedial striatum, NAc core, and NAcSh.

3.3 Experiment 3

The purpose of this experiment was to determine whether an acute social defeat stress regulated striatal 5-HT_{1B} receptor mRNA, and whether the regulation was specific to regional rostral-caudal location. In the dorsal striatum, there were no differences between control and acutely stressed groups at any level in either the dorsomedial striatum (p-values ranged from 0.42 to 0.98) or the dorsolateral striatum (p-values ranged from 0.40 to 0.98). Integrated gray levels are listed in Table 3. In addition, there were no significant group differences at any level in either the NAc core (p-values ranged from 0.17 to 0.80) or the NAcSh (p-values ranged from 0.31 to 0.76).

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4. Discussion

Social defeat is a potent and ethologically relevant stressor that can interfere with normal hedonic state and increase susceptibility to the rewarding effects of drugs of abuse [6]. Since previous data from this and other laboratories suggest that certain types of stressors, such as needle poke, social stress, and cocaine withdrawal may modulate 5-HT_{1B} receptor expression in NAc [22, 25, 26], and activation of 5-HT_{1B} receptors in the mesocorticolimbic circuit increases the rewarding effects of cocaine [20, 21, 24] and alcohol [23, 45], we investigated whether social defeat stress regulated 5-HT_{1B} mRNA levels in dorsal and ventral striatum. The results of these experiments indicate a relationship between stress duration and 5-HT_{1B} mRNA expression in the striatum, such that chronic social stress regulated 5-HT_{1B} mRNA transcript in the NAcSh and dorsolateral striatum at anatomically specific levels on the rostral-caudal axis, while an acute social stress had no effect. Although exposure to an acute tailshock stress (see supplementary material) had no significant effects on 5-HT_{1B} mRNA expression in the NAc, it did increase CRH mRNA expression in the PVN four hours after stress, indicating that the procedure induced a physiological stress response. Interestingly, there were significant correlations between CRH mRNA levels and 5-HT_{1B} mRNA expression at specific levels in both subregions of NAc, suggesting some interaction between tail shock stress, CRH mRNA, and 5-HT_{1B} expression that could be examined in more detail in future studies. It is also possible that repeated tail shock sessions or mRNA measurement at a different time point might have revealed an effect of this stress procedure on 5-HT_{1B} mRNA.

We examined chronic social defeat since it has an especially strong impact on NAc function and gene expression [46–48]. The effects of repeated social defeat on physiological and behavioral indices of chronic stress and 5-HT_{1B} mRNA regulation were tested Experiment 1. The results indicate that both intermittent and daily social defeat decreased body weight gain and induced adrenal hypertrophy, effects that have been previously described for social defeat and other chronic stress models [42, 49–52]; therefore, it appears that the social defeat procedure used in these experiments was an effective stress procedure. In addition, we observed that both intermittent and daily social defeat reduced the preference for saccharin, a non-nutritive sweet tastant that has been previously used as an index of the hedonic state of a subject [6, 7]. Additionally, we determined whether the frequency or duration of stress exposure is associated with changes in 5-HT_{1B} mRNA expression. We found that neither intermittent nor daily stress regulated receptor expression in the NAc core, but observed a trend ($p=0.08$) toward an increase in the NAcSh of animals stressed daily. The final experiment investigated whether an acute social stress (or tailshock, see supplemental data) would regulate 5-HT_{1B} transcript levels and found no social stress effect and a very modest effect in the tailshock group in the dorsomedial striatum at +1.2 mm anterior to bregma.

The NAc is an area that is involved in assessing the emotional valence of incoming stimuli, and may be important in mediating stress and fear responses. In addition to being divided into the core and shell subregions, which subserve distinct roles in behaviors such as drug seeking [53, 54], reactivation of extinguished conditioned place preference [55], and appetitive behavior [56], there is also a somatotopic organization of inputs and outputs and functional roles across the rostral-caudal extent of the NAc [57–60]. For example, Reynolds and Berridge showed that injection of muscimol, a $GABA_A$ agonist, into rostral NAcSh facilitated food reward but induced negative affect and fear behavior when infused into caudal NAcSh [32]. Therefore, anatomical precision is valuable in assessing the regulation of gene expression and behavioral impact across this gradient. We designed the third experiment to take these rostral-caudal differences in function into consideration. After chronic social defeat, we measured 5-HT_{1B} mRNA across a wide rostral-caudal span (from

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1.6 to 0.7 mm rostral to bregma) and found that in the NAcSh at the +1.0 mm level, chronic social stress dramatically upregulated 5-HT_{1B} receptor mRNA.

Although rostral NAcSh at around +1.6 mm from bregma had the greatest positive hedonic impact in the Reynolds and Berridge study, we did not detect differences in 5-HT_{1B} mRNA expression in this specific area, consistent with attenuated saccharin consumption in stressed groups. On the other hand, Reynolds and Berridge observed increased fear behavior with muscimol injection into NAcSh at 1.0 mm anterior to bregma; in the present study, chronic social stress significantly increased 5-HT_{1B} receptor mRNA expression at this locus. Since increased 5-HT_{1B} heteroreceptor activity in these neurons should result in reduced GABA release in VTA and disinhibition of dopaminergic afferents back to NAc, we would predict that increased 5-HT_{1B} expression in these neurons would also tend to inhibit their activity (similar to muscimol) and might enhance the sensitivity of these neurons to serotonin release, which is frequently elevated during aversive experiences [61]. Indeed, we observed that both rewarding and aversive responses to cocaine were enhanced by increased 5-HT_{1B} expression, depending on the timing of conditioning using the place conditioning procedure [21], although we did not examine the precise location of gene transfer along the rostral-caudal gradient in that study.

Two functional gradients may be relevant to these results. First, a dorsolateral to ventromedial gradient has been proposed to organize the striatum in regard to inputs and functional outputs; numerous subtle distinctions between core and shell have been observed [62]. The NAcSh, and especially the medial subregion that we examined here, is considered to be part of the “extended amygdala” and receives projections from limbic regions associated with stress reactivity, whereas NAc core receives more sensorimotor and “associational” inputs. For example, NAcSh receives inputs from ventral hippocampus and NAc core from dorsal hippocampus, regions of hippocampus that are associated with emotional and motivational vs. cognitive processing, respectively [63]. A second gradient from rostral to caudal has been hypothesized to contribute to rewarding vs. aversive functions, respectively. However, the somatotopic organization of inputs to rostral vs. caudal NAcSh has not been examined in great detail. In control animals, 5-HT_{1B} mRNA was relatively similar across the rostral-caudal continuum of NAc core, whereas there was a shift toward decreased 5-HT_{1B} mRNA in rostral shell and increased 5-HT_{1B} mRNA in caudal shell. In principal, if 5-HT_{1B} receptors in the NAcSh are increased, the mid-caudal striatum would be more sensitive to serotonergic input and modulation of emotional state in the face of environmental challenges. This is in agreement with the Berridge and Reynolds study that showed that the caudal NAcSh was involved in fear behaviors, whereas anterior parts were not [32].

The NAcSh is also heavily involved in modulating behaviors associated with drugs of abuse. Previous work from our group using viral mediated gene transfer to increase 5-HT_{1B} receptor levels in the NAcSh resulted in increased ethanol consumption [23, 45], changes in the pattern of consumption during both acquisition and maintenance of ethanol drinking [23], and enhancement of cocaine reward behavior that is dose dependent [21]. In addition to enhancing susceptibility to drugs of abuse, 5-HT_{1B} receptors play a role in the interaction between stress and drugs. For example, increased 5-HT_{1B} receptor expression in the NAcSh paired with a mild stressor results in facilitation of both the psychomotor activating effects of amphetamine, as well as amphetamine sensitization [22]. 5-HT_{1B} receptor regulation also occurs in response to forced cocaine withdrawal [26]. It is clear that this receptor plays an important role in drug abuse, and the current study suggests that the 5-HT_{1B} receptor in the NAcSh is modulated by stress in a regionally specific manner. Many studies demonstrate an intimate link between drug abuse/relapse and stress. Importantly, this receptor population is not responsive to acute stress, but is regulated by chronic stress exposure, which in turn

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negatively affects hedonic state. Taken together, these studies suggest that 5-HT_{1B} receptors in the mesolimbic reward pathway contribute to the hedonic and emotional impact of the environment on the organism, as well as the organism's subsequent behavior toward other environmental challenges.

In summary, these results suggest that chronic social stress increases the expression of 5-HT_{1B} receptor mRNA in the caudal NAcSh. The present results suggest the possibility that there may be a stress-associated gradient within NAcSh in the rostral-caudal dimension that further refines the role of serotonin in stress-related behavior. This same region and receptor plays a role in increased susceptibility to the rewarding effects of psychostimulants such as cocaine and amphetamine. Upregulation of this receptor in this pathway may tend to increase stress-related susceptibility to the rewarding and addictive aspects of drugs of abuse; thus, this receptor may serve as a valuable target for manipulating drug reward mechanisms in stressed individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research Highlights

1. Chronic social defeat induces hedonic deficits.
2. Chronic social defeat upregulates 5-HT_{1B} receptor mRNA expression in the NAcSh.
3. 5-HT_{1B} mRNA is regulated by stress along a rostral-caudal gradient.

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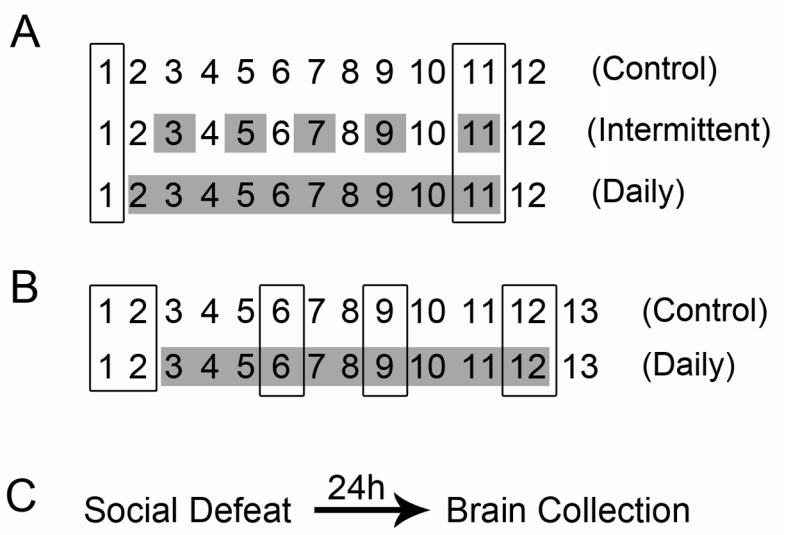

Figure 1. Schematic Diagrams of Experimental Designs

A) In Experiment 1, animals were drink trained and then defeated every other day (intermittent), every day (daily), or remained in their cages as controls. The effect of chronic stress on saccharin preference was assessed 8 hours after the last defeat episode. B) In Experiment 2, animals were defeated daily or remained in their cages as controls. Saccharin preference was assessed on two separate days before stress to determine baseline, and then at 4, 7, and 10 days after the onset of social defeat stress. Boxed days represent saccharin testing; shaded days represent social defeat. C) In Experiment 3, animals received either one social defeat stress, or remained in their cages as controls. They were then sacrificed 24 hours later. See supplemental material and methods section for tailshock stressor experimental design information.

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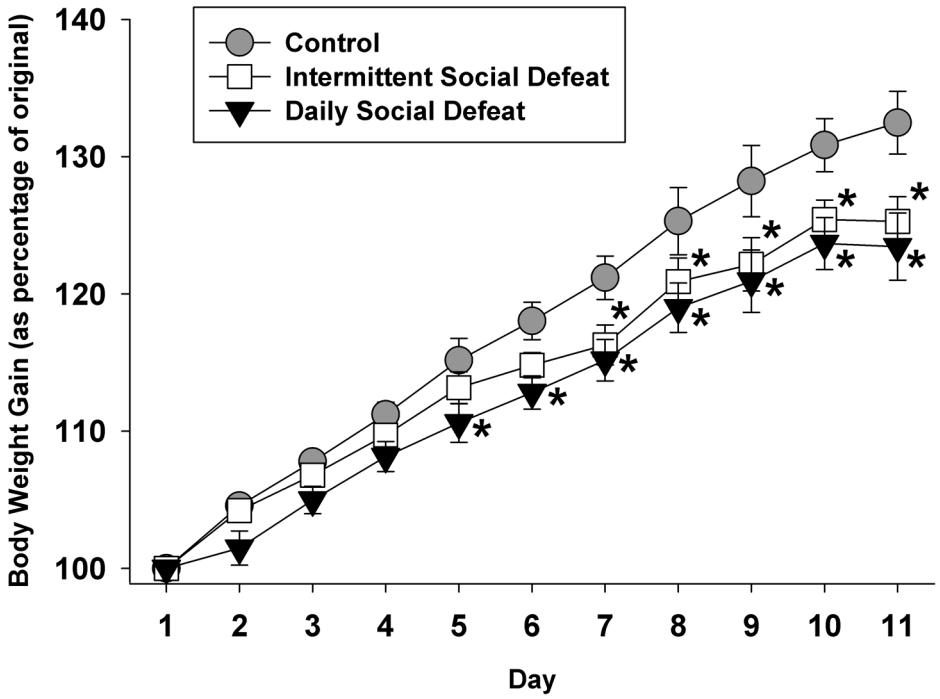

Figure 2. Stress Effects on Body Weight Gain

Both chronic and intermittent social defeat attenuated body weight gain. The daily defeated group gained significantly less body weight compared to homecage controls beginning at day 5, while intermittently defeated animals' body weight gain deficits manifested at 7 days after the onset of defeat. *=$p<0.05$, compared to control group.

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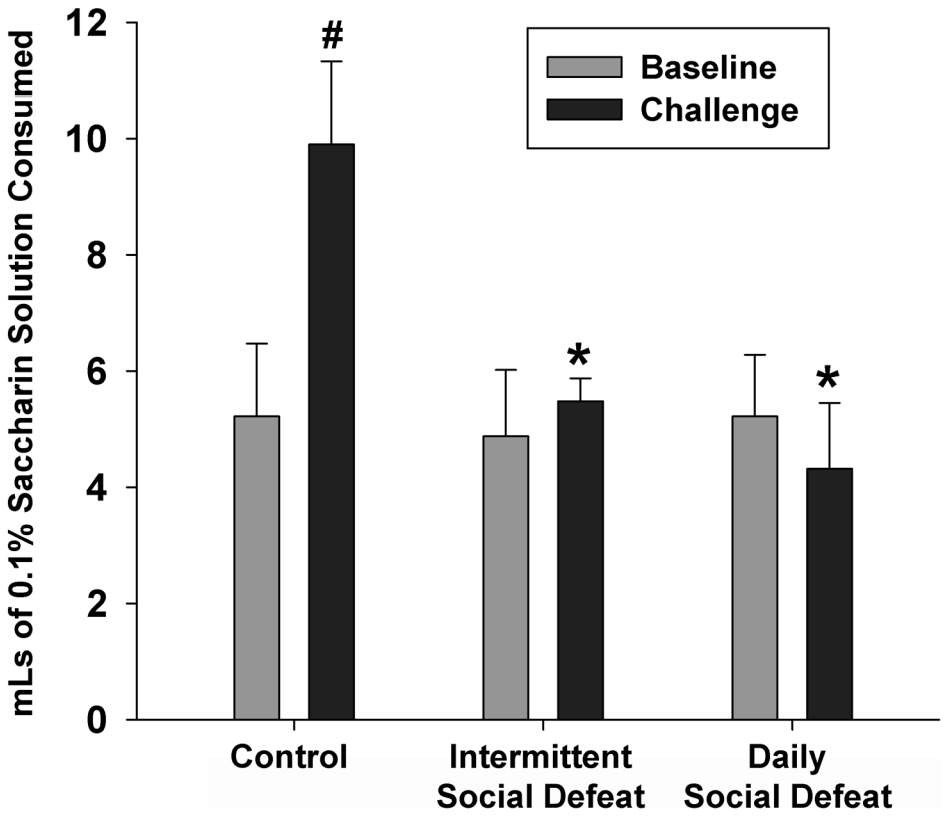

Figure 3. Stress Effects on Saccharin Preference

Both daily and intermittent stress induced hedonic deficits in the form of decreased saccharin preference. There were significant within-group effects, with the control group drinking more saccharin at the challenge, as well as a main effect of group, with both intermittent and daily stress groups drinking less saccharin than controls on the challenge test. #=p<0.05 vs. within group compared to baseline; *=p<0.05 vs. controls on challenge test.

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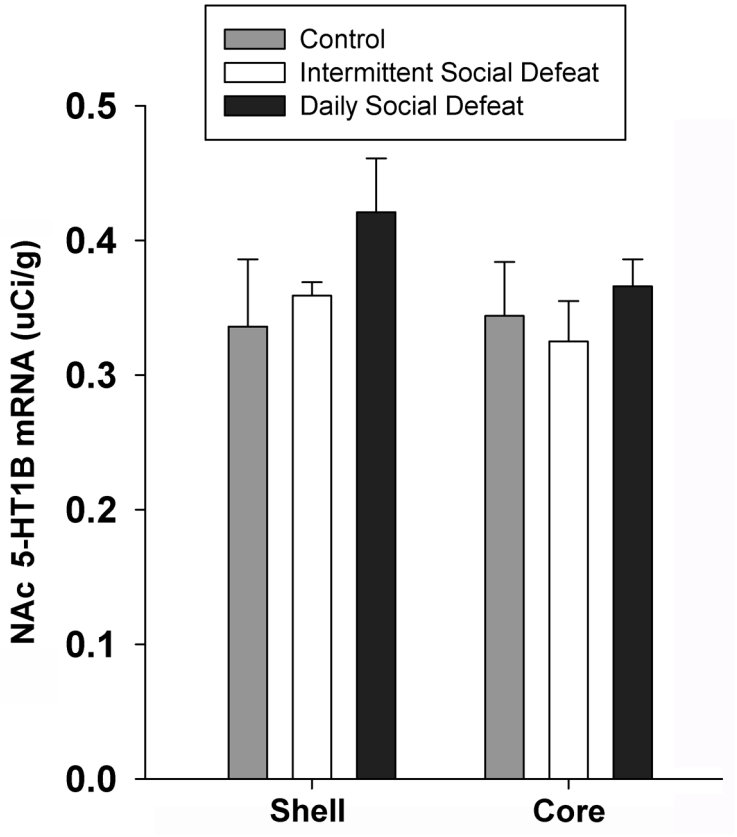

Figure 4. 5-HT$_{1B}$ mRNA Regulation After Intermittnet and Chronic Social Stress

While a single factor ANOVA revealed no significant group effects on 5-HT$_{1B}$ mRNA expression levels in either the NAcSh or NAc core, an *a priori* direct comparison revealed a trend toward increased mRNA levels in the NAcSh.

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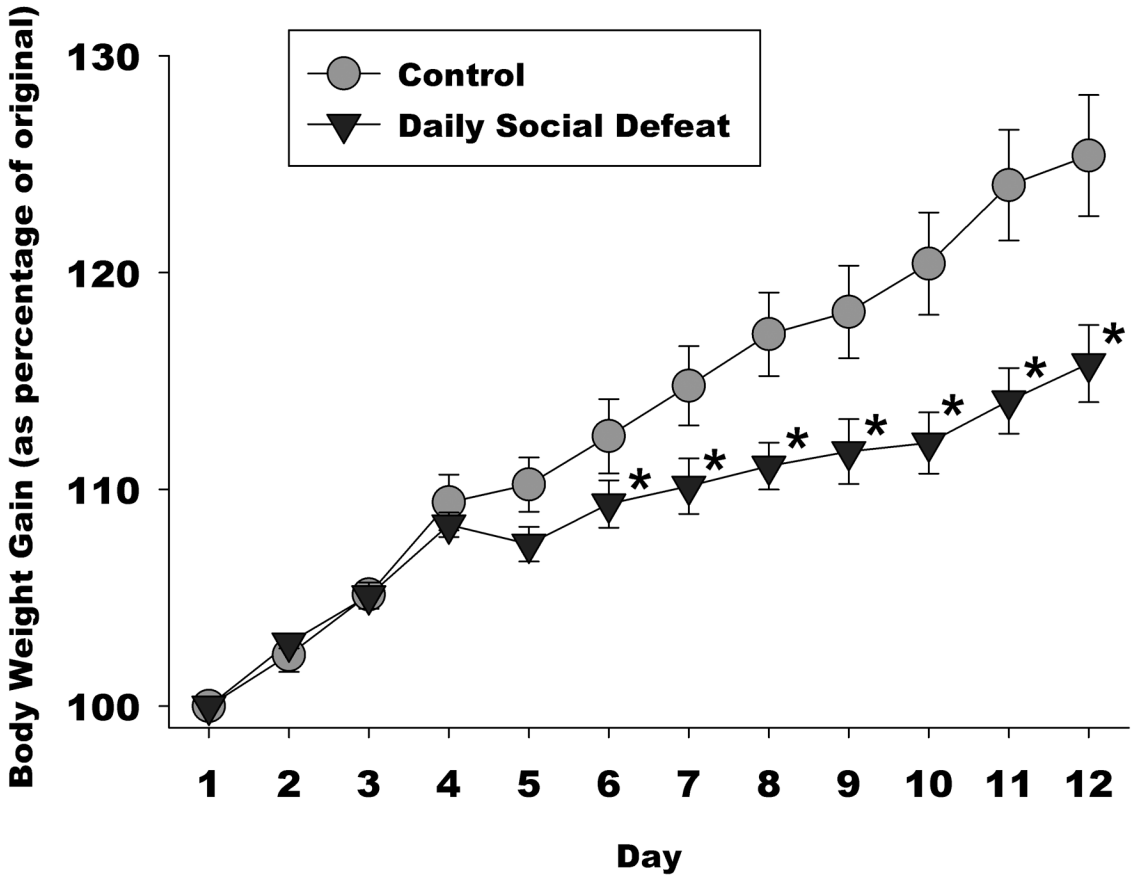

Figure 5. Chronic Social Stress Effects on Body Weight Gain

There was a main effect of group and day, as well as an interaction between group and day on body weight gain. Chronically stressed animals had attenuated body weight gain on days 6–11 compared to controls. *=$p<0.05$.

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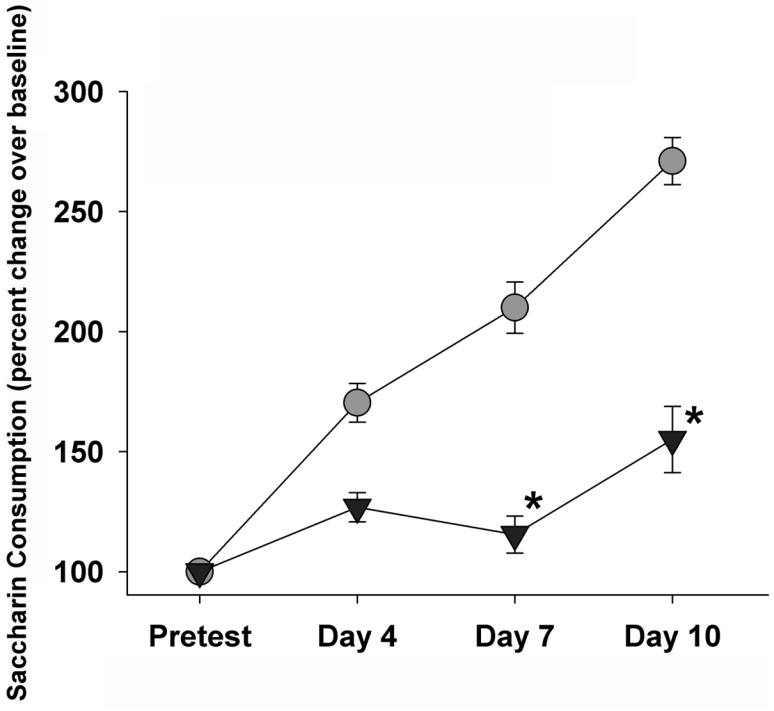

Figure 6. Chronic Stress Induces Hedonic Deficits

Chronic social defeat stress significantly induced hedonic deficits in the form of decreased saccharin preference 7 and 10 days after social defeat. *=$p<0.05$.

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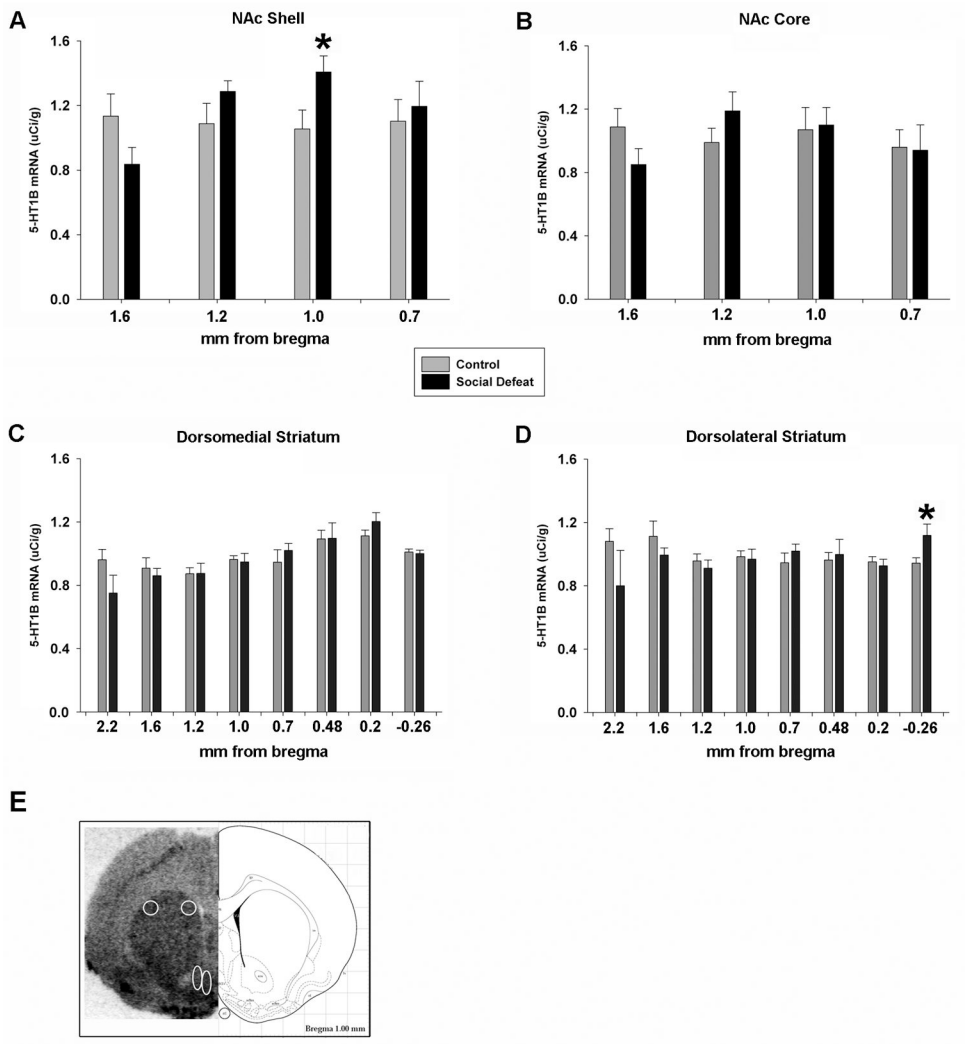

Figure 7. 5-HT$_{1B}$ mRNA Regulation After Chronic Stress

A) Ten consecutive days of social defeat stress significantly increased 5-HT$_{1B}$ mRNA signal in the NAcSh at 1.0 mm anterior to bregma. There were no differences in mRNA expression in the NAc core (B) or in the dorsomedial striatum (C). D) At −0.26 mm posterior to bregma, chronic stress increased 5-HT$_{1B}$ mRNA expression in the dorsolateral striatum. E) A representative picture of 5-HT$_{1B}$ mRNA hybridization signal from a chronially defeated animal is shown beside a panel adapted from the Paxinos and Watson rat brain atlas [64] (1.0 rostral to bregma is depicted). Striatal regions that were quantitated have been circled and include dorsolateral striatum, dorsomedial striatum, NAc core, and NAcSh. *=p<0.05.

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Table 1

Food intake during chronic social defeat stress.

	Control	Intermittent	Daily
Day 1	23.6 ± 1.23	20.9 ± 0.74	17.7 ± 2.11*
Day 2	23.5 ± 0.22	26.3 ± 0.84	23.5 ± 1.28
Day 3	25.1 ± 1.33	22.1 ± 0.96	23.8 ± 1.01
Day 4	23.3 ± 0.42	27.7 ± 0.97*,#	23.8 ± 0.87
Day 5	27.0 ± 0.82	23.1 ± 0.44	23.7 ± 0.40
Day 6	25.5 ± 0.76	27.3 ± 1.36	26.5 ± 3.15
Day 7	25.6 ± 0.67	24.9 ± 2.67	24.0 ± 1.34
Day 8	26.5 ± 0.43	23.3 ± 0.78	23.3 ± 1.05
Day 9	25.6 ± 0.92	23.4 ± 1.25	23.5 ± 1.06
Day 10	25.6 ± 0.61	24.5 ± 1.19	23.5 ± 1.02

Data are listed as ±SEM.

* $p<0.05$ vs. control;

# $p<0.05$ vs. daily stress.

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Table 2

Organ weights after intermittent and chronic social defeat stress.

	Control	Intermittent	Daily
Adrenal gland			
mg of raw weight	45.06 ± 1.78	45.01 ± 2.05	46.42 ± 1.35
mg per 100g body weight	14.81 ± 0.45	15.31 ± 0.71	16.50 ± 0.22 *
Thymus gland			
mg of raw weight	532 ± 44.5	559 ± 28.3	549 ± 28.5
mg per 100g body weight	175 ± 15.4	194 ± 12.1	193 ± 8.6

Data are listed as ±SEM.

* $p<0.05$ vs. control.

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Table 3

5-HT_{1B} mRNA signal after acute social stress, by brain region.

Region	mm From Bregma	Control	Social Defeat Stress
Nucleus Accumbens Shell			
	1.6	0.40 ± 0.07	0.36 ± 0.05
	1.2	0.42 ± 0.07	0.35 ± 0.05
	1.0	0.38 ± 0.07	0.40 ± 0.06
	0.7	0.46 ± 0.06	0.41 ± 0.05
Nucleus Accumbens Core			
	1.6	0.43 ± 0.10	0.32 ± 0.05
	1.2	0.46 ± 0.08	0.35 ± 0.04
	1.0	0.40 ± 0.08	0.38 ± 0.05
	0.7	0.40 ± 0.07	0.38 ± 0.05
Dorsolateral Striatum			
	1.6	0.57 ± 0.08	0.57 ± 0.05
	1.2	0.51 ± 0.09	0.43 ± 0.05
	1.0	0.49 ± 0.07	0.51 ± 0.06
	0.7	0.53 ± 0.09	0.45 ± 0.05
Dorsomedial Striatum			
	1.6	0.48 ± 0.08	0.48 ± 0.05
	1.2	0.43 ± 0.07	0.40 ± 0.03
	1.0	0.53 ± 0.07	0.47 ± 0.06
	0.7	0.50 ± 0.09	0.42 ± 0.05

Data are listed as ±SEM.

* $p<0.05$ vs. control.