Published in final edited form as:

Physiol Behav. 2011 August 3; 104(2): 283–290. doi:10.1016/j.physbeh.2011.02.033.

Effects of dominance status on conditioned defeat and expression of 5-HT1A and 5-HT2A receptors

Kathleen E. Morrison*, Cody L. Swallows, and Matthew A. Cooper

Department of Psychology, University of Tennessee, Knoxville, TN, 37996, USA

Abstract

Past experience can alter how individuals respond to stressful events. The brain serotonin system is a key factor modulating stress-related behavior and may contribute to individual variation in coping styles. In this study we investigated whether dominant and subordinate hamsters respond differently to social defeat and whether their behavioral responses are associated with changes in 5-HT1A and 5-HT2A receptor immunoreactivity in several limbic brain regions. We paired weight-matched hamsters in daily aggressive encounters for two weeks so that they formed a stable dominance relationship. We also included controls that were exposed to an empty cage each day for two weeks. Twenty-four hours after the final pairing or empty cage exposure, subjects were socially defeated in 3, 5-min encounters with a more aggressive hamster. Twenty-four hours after social defeat, animals were tested for conditioned defeat in a 5-min social interaction test with a non-aggressive intruder. We collected brains following conditioned defeat testing and performed immunohistochemistry for 5-HT1A and 5-HT2A receptors. We found that dominants showed less submissive and defensive behavior at conditioned defeat testing compared to both subordinates and controls. Additionally, both dominants and subordinates had an increased number of 5-HT1A immunopositive cells in the basolateral amygdala compared to controls. Subordinates also had more 5-HT1A immunopositive cells in the dorsal medial amygdala than did controls. Finally, dominants had fewer 5-HT1A immunopositive cells in the paraventricular nucleus of the hypothalamus compared to controls. Our results indicate that dominant social status results in a blunted conditioned defeat response and a distinct pattern of 5-HT1A receptor expression, which may contribute to resistance to conditioned defeat.

Keywords

social defeat; dominance; stress; resiliency; serotonin; coping style

1. Introduction

Syrian hamsters are a solitary living species in which both males and females display territorial aggression. Conditioned defeat is a model in Syrian hamsters in which an acute social defeat results in a loss of normal territorial aggression and a dramatic increase in submissive and defensive behavior in later non-aggressive social encounters. The conditioned defeat model has been used to investigate the behavioral and neurobiological

© 2011 Elsevier Inc. All rights reserved.

* corresponding author Department of Psychology Austin Peay Building University of Tennessee Knoxville, TN 37996-0900 Phone: 865-974-3412 Fax: 865-974-3330 kmcinty1@utk.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

consequences of social stress [1]. In prior experiments using this model, we have observed substantial individual variation in conditioned defeat behavior and have suspected that at least some of this variation might be due to prior dominance status, as subjects are group housed as juveniles.

Dominance status can alter an individual's stress response, although the effects of dominance status on cortisol levels have been inconsistent. For example, in some primate species, subordinates have greater stress hormone levels than dominants (long tail macaques [2]; squirrel monkeys [3]; olive baboons [4]), whereas in other species, subordinates have similar or lower stress hormone levels than dominants (rhesus macaques [5]; common marmosets [6]; cotton top tamarins [7]). Interestingly, in their meta-analysis, Abbott and colleagues [8] concluded that subordinates exhibit higher cortisol levels only when they experience higher rates of stressors and fewer opportunities for coping than do dominants. These results suggest that an important difference between dominants and subordinates may be in their style of coping with stressful events. In rodent studies, Koolhaas and colleagues have suggested that dominant rodents exhibit a proactive coping style characterized by increased reactivity with the environment, whereas subordinates exhibit a reactive coping style characterized by immobility and passivity [9, 10]. In male *Anolis* lizards, dominants have been described as adopting a proactive behavioral strategy during feeding, courtship, and social encounters, while subordinates adopt a reactive strategy [11, 12, 13]. Altogether, these studies suggest that dominant individuals might often have a temperament that allows for more effective coping, and their status might allow for more coping opportunities.

Serotonin (5-HT) is a key neurochemical that modulates aggressive behavior, coping styles, and behavioral and physiological responses to stressful events. In Syrian hamsters, 5-HT acts in the anterior hypothalamus and ventral medial hypothalamus to inhibit aggression [14, 15, 16]. The effects of 5-HT on aggression are largely mediated by 5-HT_{1A} receptors. 5-HT_{1A} receptor agonists inhibit androgen-induced aggression in hamsters and inhibit offensive aggression in highly aggressive hamsters [17, 18]. In contrast, treatment with 5-HT_{2A} receptor antagonists have been shown to reduce cocaine-induced aggression in hamsters [19]. Changes in 5-HT_{1A} and 5-HT_{2A} receptor expression are also associated with changes in aggression. High levels of aggression are associated with increased 5-HT_{1A} receptor immunoreactivity in brain regions such as the anterior hypothalamus [20]. Hamsters treated with androgens throughout adolescence are more aggressive as adults and show an increase in 5-HT_{2A} receptor immunoreactivity in the anterior hypothalamus [21]. In sum, signaling at 5-HT_{1A} receptors inhibits whereas signaling at 5-HT_{2A} receptors facilitates aggression in Syrian hamsters. Interestingly, changes in the expression of these receptors are also linked to anxiety and stress-related behavior. In mice bred for short and long attack latencies, researchers have found that highly aggressive mice (i.e. – short attack latency) display a proactive coping style and increased 5-HT_{1A} receptor binding in the hippocampus compared to low aggression mice [22, 23]. Similarly, rats bred for low anxiety-related behavior exhibit greater 5-HT_{1A} receptor mRNA in the hippocampus than do high anxiety rats [24]. Also, aversive events have been shown to alter 5-HT₂ receptor binding in several brain regions. Subordinate rats that experience chronic stress living in a visible burrow system show increased 5-HT₂ receptor binding in the parietal cortex compared to controls [25]. Also, rats that experience inescapable footshock show reduced 5-HT_{2A} receptor binding in the hippocampus [26].

Importantly, the serotonergic system has been shown to modulate at least some of the behavioral changes that characterize conditioned defeat. We have previously shown that inhibiting 5-HT cells in the dorsal raphe nucleus (DRN) by activating 5-HT_{1A} autoreceptors either before social defeat or before behavioral testing reduces both the acquisition and expression of conditioned defeat, respectively [27]. Additionally, both the acquisition and

expression of conditioned defeat can be enhanced by blocking 5-HT_{1A} autoreceptors in the DRN [27]. These results suggest that activity of 5-HT cells in the DRN contributes to both the formation and display of conditioned defeat. The basolateral amygdala (BLA) receives 5-HT projections from the DRN and is critical for the formation of conditioned defeat. The BLA is a key site for the neural plasticity underlying conditioned defeat [28, 29, 30], and we have recently demonstrated that activation of 5-HT_{1A} receptors in the BLA disrupts both the acquisition and expression of conditioned defeat (Morrison & Cooper, in press). Additionally, the BLA contains several other types of 5-HT receptors that have been linked to anxiety-related behavior, including 5-HT_{2A} and 5-HT_{2C} receptors [31, 32, 33, 34, 35]. Thus, changes in the expression of 5-HT receptors in the BLA might be capable of modifying the development of conditioned defeat.

In this study, we investigated the effects of dominance status on responses to social stress using the conditioned defeat model. Additionally, we examined differences in the expression of 5-HT_{1A} and 5-HT_{2A} receptors associated with dominance status. We hypothesized that dominant hamsters would show blunted conditioned defeat compared to subordinates and controls, and that dominants would show a distinct pattern of 5-HT_{1A} and 5-HT_{2A} receptor immunoreactivity in the BLA and other limbic brain regions.

2. Materials and Methods

2.1. Subjects

Subjects were male Syrian hamsters (*Mesocricetus auratus*) obtained from Charles River Laboratories (Wilmington, MA). We used animals that were 3-4 months old at the start of the study. Subjects were individually housed for 7-10 days prior to the experiment and remained so throughout the experiment. Older hamsters (> 6 months) were individually housed and used as resident aggressors for social defeat training. Younger hamsters (approx. 2 months) were housed in groups of four and used as non-aggressive intruders for conditioned defeat testing. All animals were housed in polycarbonate cages (12 cm × 27 cm × 16 cm) with corn cob bedding, cotton nesting materials, and wire mesh tops. Food and water were available *ad libitum*. Cages were not changed for one week prior to dominant-subordinate encounters to allow individuals to scent mark their territory. Subjects were handled during the week before dominant-subordinate encounters or empty cage exposures to habituate them to the stress of human handling. Animals were housed in a temperature controlled colony room (21 ± 2 °C) and kept on a 14:10 hr light:dark cycle. All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee.

2.2. Behavioral Protocols

2.2.1. Dominant-Subordinate Encounters—Animals (N = 24) were weight-matched in resident-intruder pairs and the same two individuals were exposed to each other in daily encounters for 14 days prior to social defeat training. We chose two weeks of pairings to be consistent with other models of chronic social stress [36]. Subjects were randomly assigned as a resident or intruder and all encounters occurred in the resident's home cage. The first encounter on day 1 was 10 min, while all subsequent encounters were 5 min. In preliminary studies, we determined that a 10 min encounter on day 1 facilitated the formation of a dominance relationship, and that 5 min encounters were sufficient to maintain the dominance relationship while reducing the chance of wounding. Dominant and subordinate animals were identified by the direction of agonistic behavior within each dyad. Dominant animals reliably displayed aggression, while subordinate animals reliably displayed submissive behavior. Each daily encounter was digitally recorded for later behavioral analysis. We quantified agonistic behavior during daily encounters using the method

described below for conditioned defeat testing. We also included empty cage controls (N =13), that were exposed to a clean, empty cage each day for either 10 min (day 1) or 5 min (days 2-14).

2.2.2. Social Defeat Training—Twenty four hours following the last dominant-subordinate encounter or last empty cage exposure, all subjects were placed in the home cages of three different resident aggressors for three separate 5-min social defeats, which occurred at 10-min intervals. Dominants often fought back against the resident aggressor during their first defeat but eventually lost and did not fight back during subsequent defeats. To correct for potential variation in the amount of aggression subjects received, we defined each defeat as starting at the resident aggressor's first attack that was accompanied by submissive behavior in a subject. Subjects lost all of their fights with the resident aggressors and never received a wound severe enough to merit removal from the study.

2.2.3. Conditioned Defeat Testing—Behavioral testing occurred 24 hours after social defeat training and consisted of a 5-minute social interaction test, during which a non-aggressive intruder was placed in the subject's home cage. Non-aggressive intruders are younger, group-housed animals that display social and nonsocial behavior during testing. One subordinate and one control subject were excluded from statistical analysis because they encountered an aggressive intruder and were attacked during testing. All testing sessions were digitally recorded and the behavior of subjects was quantified using Noldus Observer (Noldus Information Technology, Wageningen, Netherlands). We quantified the total duration of the following categories of behavior: submissive/defensive (flee, avoid, upright and side defensive postures, tail-up, stretch-attend, head flag); aggressive (chase, attack including bite, upright and side offensive postures); nonagonistic social (nose touching, sniff, approach); and nonsocial (locomotion, grooming, nesting, feeding). We also recorded the frequency of attacks, flees, and stretch-attend postures. All behavioral scoring was performed by a researcher blind to the experimental conditions of the subject. Inter-rater reliability on the duration of submissive/defensive behavior was greater than 90%.

2.3. 5-HT Immunohistochemistry

Immediately after testing, all animals were anesthetized with a cocktail of 93% sodium pentobarbital and 7% isopropyl alcohol (Sleep Away, Webster Veterinary). Then, animals were transcardially perfused with 100ml of 0.1 M PBS followed by 100ml of 4% paraformaldehyde solution. Brains were removed and soaked in 4% paraformaldehyde for 24 hours, followed by 0.1 M PBS/30% sucrose solution for 48 hours, and then were stored in cryoprotectant. A consecutive series of 40 μ m coronal sections were cut on a vibrating microtome and collected into three vials as free floating sections in cryoprotectant. The collected sections were processed for 5-HT_{1A} and 5-HT_{2A} receptor immunohistochemistry using the following protocol. All washes and incubations were conducted at room temperature unless otherwise stated. Sections were washed five times in PBS + 0.2% Triton, then were incubated for 25 min in 0.3% hydrogen peroxide and methanol solution, which was followed by 5 PBS + 0.2% Triton washes. Sections were then incubated with 0.5% goat serum (GS) in PBS + 0.2% Triton for 25 minutes before being incubated at 4°C for 48 hr in rabbit anti-serotonin-1A receptor polyclonal primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a final dilution of 1:300 in 1% GS and 0.2% Triton. Sections were then washed five times with PBS + 0.2% Triton, incubated for 60 min in biotinylated secondary anti-rabbit IgG (Vector Laboratories, Burlingame, CA) at a final dilution of 1:200 with 0.5% GS and 0.2% Triton, and washed 5 times with PBS + 0.2% Triton. Sections were then incubated in avidin-biotin-complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) with 0.2% Triton for 60 min. Following five washes with PBS + 0.2% Triton, the peroxidase reaction was visualized using a 15 min incubation in 3,3'-diaminobenzidine

(DAB tablet, Sigma-Aldrich, St. Louis, MO) and nickel dissolved in PBS. The sections were washed five times with PBS and 5 times with distilled H₂O prior to being mounted onto slides. After air-drying, sections were dehydrated using a series of alcohols, cleared with citrisolv and coverslipped using DPX (Sigma-Aldrich, St. Louis, MO).

The immunohistochemistry procedure used to detect 5-HT_{2A} receptors was identical except for the following steps. For the blocking and primary antibody steps, sections were incubated with 1% GS in PBS + 0.2% Triton for 25 minutes before being incubated at 4°C for 48 hr in rabbit anti-serotonin-2A receptor polyclonal primary antibody (Calbiochem, Gibbston, NJ, USA) at a final dilution of 1:200 in 0.2% Triton. For the secondary antibody step, sections were incubated for 60 min in biotinylated secondary anti-rabbit IgG (Vector Laboratories, Burlingame, CA) at a final dilution of 1:200 with 1% GS and 0.2% Triton.

Images of amygdala and hippocampal sections were captured at 20X magnification using an Olympus BX41 microscope, and PVN images were captured at 40X magnification. The number of 5-HT_{1A} receptor and 5-HT_{2A} receptor immunopositive cells and the relative optical density (ROD) of the 5-HT_{1A} and 5-HT_{2A} receptor immunoreactive staining were determined in select brain regions using MCID Core image analysis software (InterFocus Imaging, Cambridge, England). For 5-HT_{1A} receptors, the following brain regions were immunopositive and were quantified for cell count and relative optical density: basolateral amygdala (BLA), central amygdala (CeA), dorsal medial amygdala (dMeA), ventral medial amygdala (vMeA), and paraventricular nucleus of the hypothalamus (PVN). The CA1 layer of the dorsal hippocampus and the dentate gyrus (DG) were quantified for relative optical density although individual cell bodies could not be identified for quantification. For 5-HT_{2A} receptors, the following brain regions were quantified for relative optical density: BLA, lateral amygdala (LA), CA1, DG, and PVN. The BLA was the only brain region in which we could identify individual 5-HT_{2A} receptor immunoreactive cell bodies, and thus the only brain region in which we recorded cell counts. We measured background immunoreactivity by measuring ROD in unstained regions of the dorsal hippocampus and CeA for 5-HT_{1A} and 5-HT_{2A} receptors, respectively. For cell counts, we defined immunopositive cells as those that showed ROD 2X greater than background. For density measures, we recorded the ROD of immunopositive cells in the sampling area. Cell counts and optical density measures were limited to the area within defined sampling boxes (Fig. 1). Sample images of 5-HT_{1A} and 5-HT_{2A} receptor immunoreactivity in the BLA that represent the area within a defined sampling box are shown in Figure 2. For each brain region we quantified three sections per individual at select rostral-caudal locations.

2.8. Data Analysis

Behavioral testing and immunohistochemical data were analyzed using 1-way analysis of variance (ANOVA) with Tukey post hoc tests. ANOVAs were performed separately for each brain region, and dominance status was the independent variable. All statistical tests were two-tailed, and the α level was set at $p \leq 0.05$.

3. Results

3.1. Dominant-Subordinate Encounters

In all pairs, dominance relationships were decided during the 10 min encounter on day 1. On day 1, dominants were significantly more aggressive than subordinates ($t_{(21)} = 6.67$, $p = 0.000$). Dominant animals showed consistent levels of aggression during the subsequent 13 encounters, indicated by the duration of aggressive behavior during each encounter and the frequency of attacks directed at the subordinate, while subordinates did not show any aggression (Fig. 3). Also, dominant animals never displayed submissive behavior, whereas

subordinates showed elevated and stable submissive behavior, indicated by the duration of submissive/defensive behaviors and the frequency of fleeing during the encounter (Fig. 3). In sum, for each pair of subjects dominance status was decided on day 1, and in all cases, dominance relationships remained stable. Of 12 dominant subjects, 6 were residents and 6 were intruders in the resident-intruder paradigm. The subordinate group was comprised of 6 residents and 5 intruders. This distribution indicates that there was no effect of residency on the likelihood of becoming dominant.

3.2. Social Defeat Training

Dominant animals initially responded to social defeat training differently than did subordinate animals and controls. Due to a technical error, we only have written notes of the social defeat experience for 10 of 12 dominant subjects, 11 of 12 controls, and all 11 subordinate subjects. We found that 9 of 10 dominant subjects counter attacked the resident aggressor during the first social defeat episode, whereas 0 of 11 subordinate subjects counter attacked ($\chi^2 = 17.33$, $p < .001$ compared to dominants) and 4 of 11 empty cage control subjects counter attacked ($\chi^2 = 6.39$, $p < .05$ compared to dominants). All subjects that counter attacked submitted to the resident aggressor within 90 sec and their 5 min social defeat began upon their submission. None of the subjects counter attacked resident aggressors during the second or third social defeat episodes. Although most dominants initially fought back against resident aggressors they received the same total duration of social defeat as did subordinates and controls.

3.3. Conditioned Defeat Testing

The effects of social defeat were dependent on dominance status, such that dominant individuals showed a blunted conditioned defeat response at testing (Fig. 4). We found a main effect of dominance status on the total duration of submissive/defensive behavior displayed at testing ($F_{(2,32)} = 10.30$, $p = 0.000$). Specifically, dominant individuals displayed less submissive/defensive behavior at testing compared to both subordinates (Tukey, $p = 0.001$) and controls (Tukey, $p = 0.004$). Additionally, we found an effect of dominance status on the frequency of flees during testing ($F_{(2,23)} = 5.87$, $p = 0.007$; Dominant: 0.5 ± 0.29 ; Subordinate: 4.6 ± 1.5 ; Control: 5.8 ± 1.4). Dominant individuals displayed fewer flees at testing than both subordinates (Tukey, $p = 0.047$) and controls (Tukey, $p = 0.007$). In contrast, there was no effect of dominance status on the number of stretch attend postures during testing ($F_{(2,23)} = 0.083$, $p = 0.91$; Dominant: 1.3 ± 0.5 ; Subordinate: 1.1 ± 0.4 ; Control: 1.2 ± 0.3). We also found an effect of dominance status on the total duration of social behavior displayed at testing ($F_{(2,32)} = 8.73$, $p = 0.001$). Dominant individuals displayed more social behavior at testing compared to both subordinates (Tukey, $p = .001$) and controls (Tukey, $p = 0.014$). Dominance status did not significantly alter the duration of aggressive behavior ($F_{(2,32)} = 1.13$, $p = 0.33$) or nonsocial behavior ($F_{(2,32)} = 1.782$, $p = 0.18$).

3.4. 5-HT1A Immunohistochemistry

Social experience during dominant-subordinate encounters, rather than dominance status itself, modulated the number of 5-HT1A receptor immunopositive cells in the BLA, dMeA, and PVN (Fig. 5). We found a main effect of social experience in the BLA, such that dominants (Tukey, $p = 0.000$) and subordinates (Tukey, $p = 0.001$) had more 5-HT1A receptor immunopositive cells than did empty cage controls ($F_{(2,32)} = 12.38$, $p = 0.000$). We also found a main effect of social experience in the dMeA ($F_{(2,32)} = 5.063$, $p = 0.013$), such that subordinates had significantly more 5-HT1A receptor immunopositive cells than did empty cage controls (Tukey, $p = 0.011$), while dominants were intermediate (Tukey, $p = 0.608$, compared to subordinates; Tukey, $p = 0.081$, compared to controls). Also, we found a main effect of social experience on the number of 5-HT1A receptor immunopositive cells in

the PVN ($F_{(2,32)} = 3.804, p = 0.034$). Dominants had significantly fewer 5-HT1A receptor immunopositive cells in the PVN than did empty cage controls (Tukey, $p = 0.030$), whereas subordinates were intermediate. (Tukey, $p = 0.754$ compared to dominants; Tukey, $p = 0.142$ compared to controls)

There were no significant effects of social experience on the number of 5-HT1A receptor immunopositive cells in the CeA or vMeA ($p > 0.05$, Fig. 5). Additionally, there was no effect of social experience on the relative optical density of 5-HT1A receptor immunopositive cells in any of the brain regions quantified (Table 1).

3.5. 5-HT2A Immunohistochemistry

5-HT2A receptor immunopositive cells were larger than 5-HT1A receptor immunopositive cells (Fig. 2), which resulted in greater overlap of cells and more difficulty identifying individual cell bodies. We were able to count individual 5-HT2A receptor immunopositive cells in the BLA, although we were unable to count immunopositive cells in other regions of interest because of the dense staining. We found that social experience did not significantly alter the number of 5-HT2A receptor immunopositive cells in the BLA ($p > 0.05$, Fig. 6). 5-HT2A receptor immunopositive cells and fibers were found in the BLA, LA, CA1, DG and PVN, although social experience did not alter relative optical density of 5-HT2A receptor staining in these brain regions (Table 1).

4. Discussion

We found that dominant hamsters showed reduced submissive and defensive behavior following social defeat compared to subordinates and empty cage controls. It is noteworthy that dominants showed reduced conditioned defeat compared to controls, and that subordinates did not show increased conditioned defeat. Also, the duration of submissive and defensive behavior shown by dominants is less than the levels displayed by vehicle-treated subjects in our previous conditioned defeat studies [27, 37, 38]. Together, our results suggest that dominant social status is associated with blunted conditioned defeat. Furthermore, blunted conditioned defeat in dominant hamsters is specifically characterized by reduced submissive behavior at testing. Dominants rarely fled from non-aggressive intruders at testing, although they displayed the stretch-attend postures and a lack of territorial aggression that characterized conditioned defeat in subordinates and controls. These results suggest that dominant social status may alter neural networks that specifically control defeat-induced changes in submissive behavior. With respect to 5-HT receptors, dominants and subordinates did not differ from each other in 5-HT1A or 5-HT2A receptor immunoreactivity, which suggests that dominance status is not associated with differences in 5-HT1A or 5-HT2A receptor immunoreactivity. On the other hand, both dominants and subordinates showed changes in 5-HT1A receptor immunoreactivity in select brain regions compared to controls, which suggests that social experience during the daily encounters alters the number of cells expressing 5-HT1A receptors.

The agonistic behavior displayed by hamsters during their daily encounters indicates that they established a dominance relationship quickly and that their relationship was stable. Dominant hamsters often show decreased aggression in repeated encounters with the same opponent, although this phenomenon may be restricted to high aggressive individuals [39, 40]. In our study, dominance relationships did not function to reduce aggression in repeated encounters, and thus the experience of subordinate animals shares some similarities with chronic social defeat. However, the behavioral and neurochemical responses of subordinates were not characteristic of chronic social defeat. Subordinates did not show increased conditioned defeat compared to controls as might be expected for chronically defeated hamsters [41]. Also, subordinates did not show a down-regulation of 5-HT1A receptors

compared to controls as has been reported for subordinate rats living in a visible burrow system [25]. Thus, the chronic social defeat experienced by our subordinate subjects did not facilitate the development of conditioned defeat.

Dominant animals often adopt a proactive coping style that may modulate their responses to aversive situations. For example, dominant animals have also been characterized as natural risk takers that may be more sensitive to reward. Rats that went on to become dominant in a visible burrow system were found to display reduced anxiety behavior on an elevated-plus maze and increased operant responding for food reward following visible burrow experience [42]. In hamsters, high aggression individuals were unable to reduce their operant responding for food when switched to a delayed reward schedule designed to produce frustration, although low aggression individuals were able to adjust their responding [39]. These findings suggest that dominant hamsters might be unable to inhibit their high behavioral reactivity. In our study dominant hamsters failed to inhibit their aggression when faced with a larger opponent and they quickly counter attacked the resident aggressor. Thus, dominant hamsters appear to have responded to social defeat, at least initially, with a risky and proactive coping style. Interestingly, a proactive coping style was recently shown to reduce the effects of social defeat in rats. Animals that fought back and/or actively guarded against aggressors during social defeat displayed a blunted corticosterone response and reduced c-Fos expression in several limbic brain regions compared to animals that rarely fought back and/or guarded against aggressors [43]. One concern is that individuals that fight back against resident aggressors may not experience the same quality of social defeat as others. Although we standardized the duration of social defeat by correcting for the amount of time individuals counter attacked aggressors, we could not guarantee that dominants and subordinates received social defeats of identical quality. However, when dominants fought back, they lost dramatically in an intense fight. Also, previous research indicates that the amount of aggression received during social defeat does not correlate with conditioned defeat behavior [30, 44].

We found that dominant and subordinate hamsters showed increased 5-HT_{1A} receptor immunoreactivity in the BLA. Post-synaptic 5-HT_{1A} receptors have been shown to hyperpolarize pyramidal cells [45, 46], and therefore increased 5-HT_{1A} receptor expression should lead to reduced amygdala activity in response to 5-HT. We have recently shown that injection of a 5-HT_{1A} receptor agonist into the BLA reduces conditioned defeat [47], which is consistent with other reports indicating that pharmacological activation of 5-HT_{1A} receptors in the BLA reduces fear and anxiety-related behavior [48, 49, 50]. Nevertheless, increased 5-HT_{1A} receptor expression in the BLA can not easily explain blunted conditioned defeat in dominant hamsters because subordinates also had elevated 5-HT_{1A} receptor immunoreactivity compared to controls. It is possible that other contributing factors may have obscured changes in 5-HT receptors in the amygdala. We have previously shown that conditioned defeat is associated with increased 5-HT neurotransmission [27], and it is possible that increased 5-HT release during social defeat training or conditioned defeat testing may have produced short-term changes in 5-HT_{1A} receptor expression. Some studies suggest that elevated 5-HT neurotransmission can reduce 5-HT_{1A} receptor expression [20], although results from other studies are inconsistent [51, 52]. We believe that short-term changes in 5-HT_{1A} receptor expression are unlikely to contribute to our results for two reasons. First, elevated 5-HT neurotransmission is associated with 5-HT_{1A} receptor down-regulation whereas we found increased 5-HT_{1A} receptor expression in the amygdala. Second, our control subjects received social defeat and should have shown similar short-term changes in 5-HT_{1A} receptor expression as dominants and subordinates.

Both the BLA and MeA have been shown to regulate defensive behavior displayed during predator exposure. While the BLA integrates visual, auditory, and olfactory information to

modulate conditioned responses to a predator-related context, the MeA is critically involved in unconditioned responses to predator odor [53, 54]. The BLA is a key neural substrate controlling the formation of conditioned defeat, and recently inactivation of the MeA was shown to reduce the formation of conditioned defeat, perhaps by modulating neural plasticity in the BLA [55]. In the current study, we found that subordinates showed increased 5-HT_{1A} receptor expression in the MeA compared to controls, while dominants were intermediate. Although changes in MeA 5-HT_{1A} receptor expression may alter the salience of olfactory cues in subordinate hamsters, it does not appear to be associated with blunted conditioned defeat in dominants.

Our data on 5-HT_{1A} receptor immunoreactivity in the PVN are interesting because dominants, but not subordinates, show reduced immunoreactivity compared to controls. These data suggest the hypothesis that reduced PVN 5-HT_{1A} receptor expression in dominants might attenuate their neuroendocrine response to social defeat. Consistent with this possibility, injection of a 5-HT_{1A} receptor agonist into the PVN has been shown to increase plasma adrenocorticotropin hormone levels in rats [56, 57]. Although some studies have reported that high aggression animals show a blunted corticosterone response to social defeat [43], others have reported that dominant individuals show a more rapid stress-induced corticosterone response than do subordinates [13]. Although dominant social status may alter HPA-axis function, glucocorticoids appear to play a limited role in the development of conditioned defeat. We found that blocking glucocorticoid synthesis with injection of metyrapone prior to social defeat fails to alter the development of conditioned defeat [37]. Thus, it remains unclear how reduced 5-HT_{1A} receptor immunoreactivity in the PVN might contribute to resistance of conditioned defeat in dominant hamsters.

In the rat amygdala 5-HT_{2A} receptors have been localized to both pyramidal and nonpyramidal cells. Different 5-HT_{2A} receptor antibodies have been shown to label separate populations of amygdala neurons and the antibody we used preferentially labels nonpyramidal GABAergic neurons that co-express parvalbumin and somatostatin [31]. 5-HT has been shown to inhibit BLA neurotransmission, largely by activating 5-HT₂ receptors on GABAergic neurons [58]. Because activation of the BLA is critical for conditioned defeat [30], we predicted that resistance to conditioned defeat in dominant hamsters might have been associated with increased 5-HT_{2A} receptor immunoreactivity in the BLA. Although stressful events have been reported to alter 5-HT₂ receptor binding in a variety of brain regions [25, 26, 59], we did not observe changes in 5-HT_{2A} receptor immunoreactivity in our study. While immunohistochemistry may not be a sensitive enough technique to document subtle changes in 5-HT_{2A} receptor expression, it is also possible that stressful events might alter 5-HT₂ receptor function in the absence of changes in 5-HT₂ receptor density [60].

The work presented here indicates that dominant hamsters show resistance to conditioned defeat, although subordinates do not show increased susceptibility. Also, two weeks of daily aggressive encounters result in a distinct pattern of 5-HT_{1A} receptor expression in dominants and subordinates. The current model provides a novel approach for studying neural mechanisms underlying resistance to social stress.

Acknowledgments

We thank our team of undergraduates, most notably Daniel Curry and Carl Nix. This work was supported by NIH grant R21 MH085230 and a University of Tennessee Professional Development Award.

References

- [1]. Huhman KL. Social conflict models: Can they inform us about human psychopathology? *Hormones and Behavior*. 2006; 50:640–6. [PubMed: 16870189]
- [2]. Shively CA, Laber-Laird K, Anton RF. Behavior and physiology of social stress and depression in female cynomolgus monkeys. *Biological Psychiatry*. 1997; 41:871–82. [PubMed: 9099414]
- [3]. Steklis HD, Raleigh MJ, Kling AS, Tachiki K. Biochemical and hormonal correlates of social behavior in all-male groups of squirrel monkeys (*Saimiri sciureus*). *American Journal of Primatology*. 1986; 11:133–45.
- [4]. Sapolsky RM, Alberts S, Atlmann J. Hypercortisolism associated with social subordination or social isolation among wild baboons. *Archives of General Psychiatry*. 1997; 54:1137. [PubMed: 9400351]
- [5]. Bercovitch FB, Clarke AS. Dominance rank, cortisol concentrations, and reproductive maturation in male rhesus macaques. *Physiology & Behavior*. 1995; 58:215–21. [PubMed: 7568422]
- [6]. Saltzman W, Schultz-Darken NJ, Abbott DH. Behavioural and endocrine predictors of dominance and tolerance among female common marmosets (*Callithrix jacchus*). *Animal Behaviour*. 1996; 51:657.
- [7]. Ginther AJ, Ziegler TE, Snowdon CT. Reproductive biology of captive male cottontop tamarin monkeys as a function of social environment. *Animal Behaviour*. 2001; 61:65–78. [PubMed: 11170697]
- [8]. Abbott DH, Keverne EB, Bercovitch FB, Shively CA, Mendoza SP, Saltzman W, et al. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. *Hormones and Behavior*. 2003; 43:67–82. [PubMed: 12614636]
- [9]. Koolhaas JM, de Boer SF, Buwalda B, van Rennen K. Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. *Brain, Behavior and Evolution*. 2007; 70:218–26.
- [10]. Koolhaas JM, de Boer SF, Coppens CM, Buwalda B. Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology*. 2010; 31:307–21. [PubMed: 20382177]
- [11]. Korzan WJ, Overli O, Summers CH. Future social rank: forecasting status in the green anole (*Anolis carolinensis*). *Acta Ethologica*. 2006; 9:48–57.
- [12]. Korzan WJ, Summers CH. Behavioral diversity and neurochemical plasticity: Selection of stress coping strategies that define social status. *Brain, Behavior and Evolution*. 2007; 70:257–66.
- [13]. Ling TJ, Forster GL, Watt MJ, Korzan WJ, Renner KJ, Summers CH. Social status differentiates rapid neuroendocrine responses to restraint stress. *Physiology & Behavior*. 2009; 96:218–32. [PubMed: 18957299]
- [14]. Delville Y, Mansour K, Ferris CF. Serotonin blocks vasopressin-facilitated offensive aggression: interactions within the ventrolateral hypothalamus of golden hamsters. *Physiology & Behavior*. 1996; 59:813–6. [PubMed: 8778871]
- [15]. Ferris CF, Melloni RH, Koppel G, Perry KW, Fuller RW, Delville Y. Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. *The Journal of Neuroscience*. 1997; 17:4331–40. [PubMed: 9151749]
- [16]. Ferris CF, Stolberg T, Delville Y. Serotonin regulation of aggressive behavior in male golden hamster (*Mesocricetus auratus*). *Behavioral Neuroscience*. 1999; 113:804–15. [PubMed: 10495088]
- [17]. Cervantes MC, Biggs EA, Delville Y. Differential responses to serotonin receptor ligands in an impulsive-aggressive phenotype. *Behavioral Neuroscience*. 2010; 124:455–69. [PubMed: 20695645]
- [18]. Ricci LA, Rasakham K, Grimes JM, Melloni RH. Serotonin-1A receptor activity and expression modulate adolescent anabolic/androgenic steroid-induced aggression in hamsters. *Pharmacology Biochemistry and Behavior*. 2006; 85:1–11.
- [19]. Schwartz JJ, Morrison RL, Ricci LA, Melloni RH. Paliperidone suppresses the development of the aggressive phenotype in a developmentally sensitive animal model of escalated aggression. *Psychopharmacology*. 2009; 203:653–63. [PubMed: 19066856]

- [20]. Cervantes MC, Delville Y. Serotonin 5-HT1A and 5-HT3 receptors in an impulsive-aggressive phenotype. *Behavioral Neuroscience*. 2009; 123:589–98. [PubMed: 19485565]
- [21]. Schwartzter JJ, Ricci LA, Melloni RH. Adolescent anabolic-androgenic steroid exposure alters lateral anterior hypothalamic serotonin-2A receptors in aggressive male hamsters. *Behavioural Brain Research*. 2009; 199:257–62. [PubMed: 19110004]
- [22]. Korte SM, Meijer OC, de Kloet ER, Buwalda B, Keijser J, Sluyter F, et al. Enhanced 5-HT1A receptor expression in forebrain regions of aggressive house mice. *Brain Research*. 1996; 736:338–43. [PubMed: 8930340]
- [23]. Veenema AH, Cremers TI, Jongsma ME, Steenbergen PJ, de Boer SF, Koolhaas JM. Differences in the effects of 5-HT1A receptor agonists on forced swimming behavior and brain 5-HT metabolism between low and high aggressive mice. *Psychopharmacology*. 2005; 178:151–60. [PubMed: 15448978]
- [24]. Keck ME, Sartori SB, Welt T, Muller MB, Ohi F, Holsboer F, et al. Differences in serotonergic neurotransmission between rats displaying high or low anxiety/depression-like behaviour: effects of chronic paroxetine treatment. *Journal of Neurochemistry*. 2005; 92:1170–9. [PubMed: 15715667]
- [25]. McKittrick CR, Blanchard DC, Blanchard RJ, McEwen BS, Sakai RR. Serotonin receptor binding in a colony model of chronic social stress. *Biological Psychiatry*. 1995; 37:383–93. [PubMed: 7772647]
- [26]. Wu J, Kramer GL, Kram M, Steciuk M, Crawford IL, Petty F. Serotonin and learned helplessness: a regional study of 5-HT1A, 5-HT2A receptors and the serotonin transport site in rat brain. *Journal of Psychiatric Research*. 1999; 33:17–22. [PubMed: 10094235]
- [27]. Cooper MA, McIntyre KE, Huhman KL. Activation of 5-HT1A autoreceptors in the dorsal raphe nucleus reduces the behavioral consequences of social defeat. *Psychoneuroendocrinology*. 2008; 33:1236–47. [PubMed: 18692968]
- [28]. Day DE, Cooper MA, Markham CM, Huhman KL. NR2B subunit of the NMDA receptor in the basolateral amygdala is necessary for the acquisition of conditioned defeat in Syrian hamsters. *Behavioural Brain Research*. 2010 In Press.
- [29]. Jasnow AM, Cooper MA, Huhman KL. N-methyl-D-aspartate receptors in the amygdala are necessary for the acquisition and expression of conditioned defeat. *Neuroscience*. 2004; 123:625–34. [PubMed: 14706775]
- [30]. Jasnow AM, Huhman KL. Activation of GABA(A) receptors in the amygdala blocks the acquisition and expression of conditioned defeat in Syrian hamsters. *Brain Research*. 2001; 920:142–50. [PubMed: 11716820]
- [31]. McDonald AJ, Mascagni F. Neuronal localization of 5-HT type 2A receptor immunoreactivity in the rat basolateral amygdala. *Neuroscience*. 2007; 146:306–20. [PubMed: 17331657]
- [32]. Pompeiano M, Palacios J, Mengod G. Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT1A and 5-HT2C receptors. *Molecular Brain Research*. 1994; 23:163–78. [PubMed: 8028479]
- [33]. Campbell BM, Merchant KM. Serotonin 2C receptors within the basolateral amygdala induce acute fear-like responses in an open-field environment. *Brain Research*. 2003; 993:1–9. [PubMed: 14642825]
- [34]. Cornelio AM, Nunes-de-Souza RL. Anxiogenic-like effects of mCPP microinfusions into the amygdala (but not dorsal or ventral hippocampus) in mice exposed to elevated plus-maze. *Behavioural Brain Research*. 2007; 178:82–9. [PubMed: 17207863]
- [35]. Weisstaub NV, Zhou M, Lira A, Lambe E, Gonzalez-Maeso J, Hornung JP, et al. Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. *Science*. 2006; 313:536–40. [PubMed: 16873667]
- [36]. Blanchard DC, Spencer RL, Weiss SM, Blanchard RJ, McEwen BS, Sakai RR. Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. *Psychoneuroendocrinology*. 1995; 20:117–34. [PubMed: 7899533]
- [37]. Cooper MA, Huhman KL. Blocking corticotropin-release factor-2 receptors, but not corticotropin-releasing factor-1 receptors of glucocorticoid feedback, disrupts the development of conditioned defeat. *Physiology & Behavior*. 2010; 101:527–32. [PubMed: 20705077]

- [38]. Cooper MA, Karom M, Huhman KL, Albers HE. Repeated agonistic encounters in hamsters modulate AVP V1a receptor binding. *Hormones and Behavior*. 2005; 48:545–51. [PubMed: 15935353]
- [39]. David J, Cervantes MC, Trosky K, Salinas J, Delville Y. A neural network underlying individual differences in emotion and aggression in male golden hamsters. *Neuroscience*. 2004; 126:567–78. [PubMed: 15183506]
- [40]. Ferris CF, Axelson J, Shinto L, Albers HE. Scent marking and the maintenance of dominant/subordinate status in male golden hamsters. *Physiology & Behavior*. 1987; 40:661–4. [PubMed: 3671532]
- [41]. Potegal M, Huhman KL, Moore T, Meyerhoff J. Conditioned defeat in the Syrian golden hamster (*Mesocricetus auratus*). *Behavioral and Neural Biology*. 1993; 60:93–102. [PubMed: 8117243]
- [42]. Davis JF, Krause EG, Melhorn SJ, Sakai RR, Benoit SC. Dominant rats are natural risk takers and display increased motivation for food reward. *Neuroscience*. 2009; 162:23–30. [PubMed: 19393296]
- [43]. Walker FR, Masters LM, Dielenberg RA, Day TA. Coping with defeat: acute glucocorticoid and forebrain responses to social defeat vary with defeat episode behaviour. *Neuroscience*. 2009; 162:244–53. [PubMed: 19393295]
- [44]. Solomon MB, Karom M, Norvelle A, Markham CA, Erwin WD, Huhman KL. Gonadal hormones modulate the display of conditioned defeat in male Syrian hamsters. *Hormones and Behavior*. 2009; 56:423–8. [PubMed: 19651128]
- [45]. Palchoudhuri M, Flugge G. 5-HT1A receptor expression in pyramidal neurons of cortical and limbic brain regions. *Cell and Tissue Research*. 2005; 321:159–72. [PubMed: 15947971]
- [46]. Stein C, Davidowa H, Albrecht D. 5-HT(1A) receptor-mediated inhibition and 5-HT(2) as well as 5-HT(3) receptor-mediated excitation in different subdivisions of the rat amygdala. *Synapse*. 2000; 38:328–37. [PubMed: 11020236]
- [47]. McIntyre, KE.; Cooper, MA. 2008 Neuroscience Meeting Planner. Society for Neuroscience; Washington, DC: 2008. Infusion of a 5-HT1a receptor agonist into the basolateral amygdala reduces conditioned defeat. Program No. 95.18. Online
- [48]. Gorenink L, Joordens RJ, Hijzen TH, Dirks A, Olivier B. Infusion of flesinoxan into the amygdala blocks the fear-potentiated startle. *Neuroreport*. 2000; 11:2285–8. [PubMed: 10923686]
- [49]. Li X, Inoue T, Abekawa T, Weng S, Nakagawa S, Izumi T, et al. 5-HT1A receptor agonist affects fear conditioning through stimulations of the postsynaptic 5-HT1A receptors in the hippocampus and amygdala. *European Journal of Pharmacology*. 2006; 532:74–80. [PubMed: 16460727]
- [50]. Zangrossi H Jr, Viana MB, Graeff FG. Anxiolytic effect of intra-amygdala injection of midazolam and 8-hydroxy-2(di-*n*-propylamino)tetralin in the elevated T-maze. *European Journal of Pharmacology*. 1999; 369:267–70. [PubMed: 10225362]
- [51]. Spurlock G, Buckland P, O'Donovan M, McGuffin P. Lack of effect of antidepressant drugs on the levels of mRNAs encoding serotonergic receptors, synthetic enzymes and 5HT transporter. *Neuropharmacology*. 1994; 33:433–40. [PubMed: 7984281]
- [52]. Carli M, Afkhami-Dastjerdian S, Reader TA. [3H]8-OH-DPAT binding and serotonin content in rat cerebral cortex after acute fluoxetine, desipramine, or pargyline. *Journal of Psychiatry and Neuroscience*. 1996; 21:114–22. [PubMed: 8820177]
- [53]. Martinez RC, Carvalho-Netto EF, Ribeiro-Barbosa ER, Baldo MVC, Canteras NS. Amygdalar roles during exposure to a live predator and to a predator-associated context. *Neuroscience*. 2010 In Press, Corrected Proof.
- [54]. Blanchard DC, Canteras NS, Markham CA, Pentkowski NS, Blanchard RJ. Lesions of structures showing FOS expression to cat presentation: effects on responsibility to a Cat, Cat odor, and nonpredator threat. *Neuroscience and Biobehavioral Reviews*. 2005; 29:1243–53. [PubMed: 16084591]
- [55]. Markham CM, Huhman KL. Is the medial amygdala part of the neural circuit modulating conditioned defeat in Syrian hamsters? *Learning and Memory*. 2008; 15:6–12. [PubMed: 18174368]

- [56]. Osei-Owusu P, James A, Crane J, Scroin KE. 5-Hydroxytryptamine 1A receptors in the paraventricular nucleus of the hypothalamus mediate oxytocin and adrenocorticotropin hormone release and some behavioral components of the serotonin syndrome. *The Journal of Pharmacology and Experimental Therapeutics*. 2005; 313:1324–30. [PubMed: 15743927]
- [57]. Jorgensen H, Knigge U, Kjaer A, Moller M, Warberg J. Serotonergic stimulation of corticotropin-releasing hormone and pro-opiomelanocortin gene expression. *Journal of Neuroendocrinology*. 2002; 14:788–95. [PubMed: 12372003]
- [58]. Rainnie DG. Serotonergic modulation of neurotransmission in the rat basolateral amygdala. *Journal of Neurophysiology*. 1999; 82:69–85. [PubMed: 10400936]
- [59]. Schiller L, Donix M, Jahkel M, Oehler J. Serotonin 1A and 2A receptor densities, neurochemical and behavioural characteristics in two closely related mice strains after long-term isolation. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2006; 30:492–503. [PubMed: 16412547]
- [60]. Benekareddy M, Goodfellow NM, Lambe EK, Vaidya VA. Enhanced function of prefrontal serotonin 5-HT(2) receptors in a rat model of psychiatric vulnerability. *Journal of Neuroscience*. 2010; 30:12138–50. [PubMed: 20826676]
- [61]. Morin, LP.; Wood, RI. *A Stereotaxic Atlas of the Golden Hamster Brain*. Academic Press; San Diego: 2001.

Figure 1.

The diagrams indicate the location of brain regions selected for quantification of 5-HT1A and 5-HT2A receptor immunoreactivity. The diagrams were modified from a hamster brain atlas and values indicate the distance from bregma [61]. The box sizes used for quantification are as follows: $330\ \mu\text{m} \times 439\ \mu\text{m}$ (BLA, CeA, LA, dMeA, vMeA, CA1, and DG); $165\ \mu\text{m} \times 219\ \mu\text{m}$ (PVN).

Figure 2. Representative photomicrographs of a hamster coronal brain section used for immunohistochemical analyses. a) Photomicrograph of tissue from the BLA labeled for 5-HT1A receptor immunoreactivity. b) Photomicrograph of tissue from the BLA that was labeled for 5-HT2A receptor immunoreactivity.

Figure 3.

Agonistic behavior during daily encounters. Encounters were 10 min on Day 1 and 5 min on Days 2-14. a) Duration (mean $\pm$ SE) of aggressive behavior displayed by dominants ($N = 12$) and subordinates ($N = 11$) during fourteen days of encounters. Dominant animals showed consistent levels of aggression throughout all encounters, while subordinates showed no aggression. b) Duration (mean $\pm$ SE) of submissive and defensive behavior displayed by dominants and subordinates throughout the encounters. Subordinate animals showed consistent levels of submissive and defensive behavior, while dominants showed none. c) Number (mean $\pm$ SE) of attacks during each daily encounter. Subordinates never attacked, while dominants showed consistent levels of attacking. d) Number (mean $\pm$ SE) of flees during each daily encounter. Subordinates showed consistent levels of fleeing, while dominants never fled.

Figure 4.

Durations (mean $\pm$ SE) of submissive, aggressive, social and nonsocial behaviors are shown during a 5 min test with a non-aggressive intruder. All subjects received social defeat training 24h prior to testing. Dominant (N = 12) and subordinate (N = 11) individuals experienced 14 days of dyadic encounters prior to testing. Empty cage control (N = 12) individuals were exposed to a clean, empty cage daily for 14 days. An asterisk (*) indicates $p < 0.05$ compared to subordinates and empty cage controls.

Figure 5.

Number (mean ± SE) of 5-HT_{1A} receptor immunopositive cells measured in several brain regions following conditioned defeat testing. An asterisk (*) indicates $p < 0.05$ compared to empty cage controls. Sample sizes ranged from 10-12 individuals per group, because of variation in tissue quality.

Figure 6. Number (mean $\pm$ SE) of 5-HT_{2A} receptor immunopositive cells measured in the BLA following conditioned defeat testing. There were no significant differences between the groups. Dominant (N = 12), Subordinate (N = 11), Empty Cage (N = 12).

Table 1

Mean $\pm$ SEM relative optical density (ROD) of 5-HT1A and 5-HT2A receptor immunopositive cells. Dominance status did not significantly alter ROD in any of the brain regions surveyed.

	Dominant (N = 10-12)	Subordinate (N = 10-11)	Empty Cage (N = 10-12)	<i>p</i>
5-HT1A Immunopositive Regions				
Hippocampus – CA1	0.421 $\pm$ 0.012	0.409 $\pm$ 0.015	0.387 $\pm$ 0.011	ns
Hippocampus – DG	0.450 $\pm$ 0.021	0.450 $\pm$ 0.020	0.406 $\pm$ 0.014	ns
BLA	0.377 $\pm$ 0.004	0.374 $\pm$ 0.007	0.364 $\pm$ 0.005	ns
CeA	0.374 $\pm$ 0.005	0.372 $\pm$ 0.007	0.361 $\pm$ 0.005	ns
dMeA	0.394 $\pm$ 0.004	0.392 $\pm$ 0.008	0.379 $\pm$ 0.006	ns
vMeA	0.399 $\pm$ 0.005	0.398 $\pm$ 0.007	0.383 $\pm$ 0.007	ns
PVN	0.391 $\pm$ 0.008	0.391 $\pm$ 0.007	0.391 $\pm$ 0.005	ns
5-HT2A Immunopositive Regions				
Hippocampus – CA1	0.383 $\pm$ 0.015	0.393 $\pm$ 0.012	0.385 $\pm$ 0.014	ns
Hippocampus – DG	0.353 $\pm$ 0.033	0.358 $\pm$ 0.010	0.341 $\pm$ 0.008	ns
BLA	0.366 $\pm$ 0.005	0.382 $\pm$ 0.011	0.373 $\pm$ 0.007	ns
LA	0.365 $\pm$ 0.006	0.385 $\pm$ 0.011	0.365 $\pm$ 0.012	ns
PVN	0.530 $\pm$ 0.017	0.537 $\pm$ 0.020	0.505 $\pm$ 0.018	ns

Note: Sample sizes vary because some individuals were excluded from data analysis due to poor tissue quality.