RATS EXHIBIT BEHAVIORAL DESPAIR AND HORMONAL ALTERATIONS AFTER SOCIAL DEFEAT STRESS: IMPLICATIONS FOR MAJOR DEPRESSION

By

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To my loving husband (Justin),
my wonderful parents (Linda, Kenneth, and Donna),
and my brothers and sisters (Kim, Rick, Minet, Roger, and Jay).
Thank you for your uninterrupted love and support.

Also to my extended family (both biological and in-law) and my friends.
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Chronic emotional stress plays a pivotal role in the origin of many psychiatric disorders, including major depression. Accordingly, we investigated the behavioral, hormonal, and glandular effects of both repeated and acute emotional stress using the social defeat model in rats. We compared one week of daily social defeat exposure to one month of intermittent exposure to examine the effectiveness of massed versus intermittent stressors.

In two experiments, naïve male intruder rats were each exposed to dominant male resident rats. Each resident and intruder pair was allowed to interact for 5 minutes or until the intruder exhibited submissive supine posture three times. The intruder was then briefly removed, placed into a protective wire mesh cage, and returned to the resident's cage until a total of 10 minutes elapsed from initial entry. Additional control rats were not exposed to social defeat stress. For the first experiment, the intruder rats were killed 10 minutes, 30 minutes, or 24 hours after the last social defeat session and plasma

corticosterone and adrenocorticotropic hormone concentrations were assayed. For the second experiment, the previously-stressed rats were tested with a 15-minute Porsolt forced swim test.

The intruder rats exhibited more freezing behavior and less exploratory locomotion across consecutive social defeat sessions, resembling the loss of interest found in depressed patients. Exposure to social defeat stress produced significant elevations in circulating hormones 10 minutes and 30 minutes after the session, when compared with concentrations in the control rats. The repeatedly stressed rats also exhibited higher basal concentrations of circulating corticosterone 24 hours later, mimicking the augmented circulating hormones found in clinically depressed patients. These results were evident after six daily exposures; however, basal hormone concentrations were not significantly elevated with the extended regimen of one social defeat session every 72 hours. Inconsistent thymus involution in the chronically stressed rats in both regimens suggests that a longer, more intense daily stress regimen may be necessary to alter glandular masses. Exposure to chronic social defeat stress also produced a significant increase in total immobility time during the forced swim test when compared with the immobility times for the rats that were exposed to a single acute social defeat session and with the immobility times for the rats that were not exposed to social defeat stress, thus representing behavioral despair in the chronically stressed animals. Overall, the behavioral, hormonal, and glandular alterations that occurred after repeated social defeat stress resemble some of the symptoms of major depression in humans.

CHAPTER 1
INTRODUCTION

Annually, approximately 6.6% of the national adult population suffers from depression (Kessler et al., 2003). It is a wide-spread illness that interferes with the ability to eat, sleep, work, and enjoy formerly pleasurable activities. The economic impact of this devastating disorder is high, but the cost in human distress cannot be estimated.

The 4th Edition of the Diagnostic and Statistical Manual of Mental Disorders (1994) defines depression according to the following criteria, with at least five of the symptoms present on a daily basis for at least 2 weeks: depressed mood, loss of interest or pleasure, significant weight loss or gain, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue, undue guilt and/or feelings of worthlessness, indecisiveness, recurrent thoughts of death, and significant distress or impairment in social or occupational functioning. These symptoms must represent a change from the individual's normal level of interpersonal function.

Major depression is also clinically characterized by altered hormonal function stemming from ongoing elevations in overall organism distress. It has been well established that chronic emotional stress plays a pivotal role in the genesis of many psychiatric disorders such as depression (for review, see Agid et al., 2000). Chronic stress weighs on the physiological systems that maintain homeostasis and produces changes in the operating limits of those hormonal systems. Allostatic load, or the strain from the elevated activity of systems under major stress, can predispose an animal to many psychiatric disorders, including depression (McEwen and Stellar, 1993). The

concept of allostatic load suggests that there is a fixed state in which enduring environmental challenges are balanced by a hormonal response that is raised above normal, basal levels. Patients with major depression, regardless of age, show higher 24-hour average cortisol levels when compared to normal subjects (Linkowski et al., 1985). Additionally, depressed patients reach the nadir of their daily cortisol cycle two to three hours before control subjects (Pfohl et al., 1985). This imbalance between activation and recovery of the stress response is implicated in the inability to maintain homeostasis, thus leading to neuroendocrine maladjustment and heightened risk for depression (De Kloet, 2003).

Stressful stimuli are categorized into two descriptive classes. Systemic stressors, such as exposure to heat or cold, present immediate threats to somatic homeostasis while processive stressors emphasize higher level cognitive processing (Herman and Cullinan, 1997). Common processive stressors include instability in the social hierarchy and loss of environmental control. In accordance with the emotional nature of processive stress, it is particularly implicated in a variety of psychiatric disorders including depression (for review, see Anisman and Matheson, 2005).

Inputs from the brainstem (if the stressor is systemic in nature) and cortical and limbic structures (if the stressor is processive in nature) converge at the paraventricular nucleus (PVN) of the hypothalamus where parvocellular neurons project to the median eminence. From there, corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) are released into the hypophyseal portal circulation, stimulating adrenocorticotropic hormone (ACTH) release from the anterior pituitary corticotrope cells into the general circulation. The release of ACTH then stimulates the synthesis and

release of glucocorticoids from the adrenal cortex. Cortisol is the main glucocorticoid in humans and corticosterone (CORT) is the main glucocorticoid in rats. The elevated circulating levels of glucocorticoids decrease the further activity of the HPA axis through exertion of negative feedback on neurosecretory cells of the hypothalamus and corticotrope cells of the pituitary (for review, see Whitnall, 1993).

Altered regulation of hormonal activity in depressed patients is thought to be a result of increased activity of specific CRH-containing neurons in the PVN. The average total number of such neurons is up to four times higher in depressed patients when compared to normal control subjects. Also, co-localization of AVP in CRH-expressing neurons has been indicated as an index for stress-activated neuronal activity. The average number of neurons co-expressing both CRH and AVP in depressed patients is up to three times higher than those for normal subjects. These results suggest that increased expression of CRH- and AVP-containing neurons in the PVN may cause at least a fraction of the collective symptomatology of depression (Raadsheer et al., 1994).

When physically or emotionally stressed, non-human animals endure physiological responses that lead to behavioral and hormonal impairment which may be fundamentally similar to the impairment seen in human stress-induced psychopathology. Behavioral responses to stressful stimuli (including increased drug-taking propensity, decreased performance in learning tasks, sleep disturbances, and unsocial behavior) have been observed in a variety of species (for review, see Amiel-Tison et al., 2004). Also, there is significant evolutionary homology in stress-regulating peptides, such as CRH and CRH-related molecules (Chang and Hsu, 2004). Thus, the use of an animal model for

processive stress that produces both behavioral and hormonal effects is a logical approach to the study of human stress-induced disorders.

The effects of chronic and acute processive stress have been studied in rats (Simpkiss and Devine, 2003). Experimentally naïve rats were exposed to a chronic variable stress (CVS) regimen of twice daily stressors for fifteen days. The stressors included novel environment, switched cage mates, forced swim, light open field, intermittent white noise, and intermittent footshock, administered on a random intermittent schedule. The CVS regimen was unsuccessful in producing elevations in basal circulating concentrations of ACTH and CORT, or in hormonal response to an acute stressor. The rats showed a blunted ACTH response, but no altered CORT response.

Another model for emotional stress, social defeat, has also produced significant elevations in circulating CORT during and after acute and repeated exposure to the stressful stimulus (Sgoifo et al., 1996). The procedure, developed by Miczek (1979), is designed to model social stress. A male "intruder" rat is exposed to social stress when it is placed into the home cage of a larger male "resident" rat. The resident rat exhibits dominant behavior toward the intruder rat by displaying assertive posture, standing over the intruder. The intruder submits by displaying supine posture, positioned beneath the resident.

The further effects of repeated social defeat stress on behavioral and hormonal (HPA axis) responses have been studied in rats (Lopes and Devine, 2004). In a preliminary study, a repeatedly stressed group of intruder rats showed significantly elevated circulating CORT concentrations 24 hours after their final social defeat session,

when compared to the concentrations in the unstressed control animals. This suggests an enduring change in the circadian regulation of the HPA axis following chronic social defeat. Overall, the effects of six days of social defeat stress exceeded the results attained with the fifteen days of CVS previously described.

The brain sites activated by social defeat have been studied using *c-fos* immunohistochemistry. The immediate-early gene (IEG) *c-fos* is expressed in many cells in the brain, but typically at very low basal levels. *C-fos* and other IEGs are intracellular signaling mechanisms that regulate gene transcription and expression of various neuropeptides and trophic molecules in response to stress (for review, see Sabban and Kvetnansky, 2001). Various stressful stimuli can initiate increased levels of *c-fos* mRNA expression, lasting for minutes to hours. For instance, social defeat produces elevated *c-fos* expression in limbic, limbic-associated, and brainstem sites in both hamsters and rats one hour after a single defeat session (Kollack-Walker et al., 1997; Martinez et al., 1998). These results point to the brain structures that are important in the processing of emotionally stressful events. However, after repeated social defeat sessions in rats, the pattern of neuronal activity was modified, despite the fact that intruder submissive behavior persisted across trials. *C-fos* mRNA expression endured for many of these limbic and brainstem nuclei, while other limbic and brainstem regions exhibited a decrease in the social defeat-induced *c-fos* mRNA expression.

The Porsolt swim test, originally described by Porsolt and colleagues (1978), is the most commonly utilized behavioral test for screening antidepressant treatments in rats and has been used to evaluate the behavioral effects of stress exposure. Immobility during the inescapable swim is measured as an indicator of behavioral despair or

depressive-like behavior. Regimens of chronic stress or repeated administration of gluccocorticoids increase the amount of time an animal spends immobile, decrease the latency to immobility, and decrease the amount of time engaged in active swimming (Molina et al., 1994; Johnson et al., 2006). Antidepressant drugs, on the other hand, ameliorate these effects. For example, desipramine (a tricyclic antidepressant) and fluoxetine (a selective serotonin reuptake inhibitor) reduce immobility and increase the amount of time a stressed animal will struggle to escape the forced swim (Molina et al., 1994; Lucki et al., 2001).

Based on the results of investigations using social defeat stress and the Porsolt swim test, we have begun to further characterize the social defeat model of emotional stress in rats. We have evaluated the impact of both acute and repeated social defeat stress on regulation of ACTH and CORT at various times after the stressor. We have also compared six daily social defeat sessions to one month of stress every third day to examine the effectiveness of massed and intermittent stress exposure. In addition, we exposed socially defeated rats to the Porsolt swim test in order to characterize the enduring behavioral effects of social defeat stress.

CHAPTER 2
METHODS

Animals

Ninety male Long-Evans (LE) rats and twelve female LE rats were purchased from Harlan Co. of Indianapolis, IN. Twenty of the male Long Evans (LE) rats (225-250 g) were used as unhandled controls. Fifty-eight of the male LE rats (225-250 g) were used as intruders. Twelve of the male LE rats (300-325 g) were used as residents and the twelve female LE rats (225-250 g) were used as housing mates for the residents. The weight ranges indicate weights at the time the rats were purchased. The intruders weighed 275-325 g and the residents weighed 500-800 g at the time of the experiments.

Six of the unhandled control rats were used in Experiment 1a, six were used in Experiment 1b, and eight were used in Experiment 2. Thirty of the intruder rats were used in Experiment 1a, twelve were used in Experiment 1b, and sixteen were used in Experiment 2. The resident males and the females were used in multiple experiments.

The rats were housed in polycarbonate cages (43 x 21.5 x 25.5 cm) with sex-matched and weight-matched pairings for five to seven days of acclimation to a 12hr/12hr light/dark cycle (lights on at 7:00a.m.). Standard chow (LabDiet 5001) and tap water were available *ad libitum*. Temperature and humidity in the housing facility were controlled to 22.72°C ± 0.94°C and 54.1% ± 14.6%, respectively.

The intruder rats were exposed to social defeat stress and remained pair-housed throughout the experiment. The control rats were not exposed to social defeat stress and were pair-housed throughout the experiment. The resident rats were vasectomized,

singly-housed, and given 10 days to recover from the surgery. The residents were then pair-housed in a separate housing room, each with a female, for 2 weeks prior to and then throughout the experiment. All procedures were pre-approved by the Institutional Animal Care and Use Committee at the University of Florida and conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

Surgical Procedures

After a week of acclimation to the housing facility, the resident rats were anaesthetized with ketamine:xylazine (50 mg/kg:5 mg/kg, i.p.). Sedation was verified by testing the pedal withdrawal reflex 10 min after injection and every 10 min during the procedure. After sedation was verified, each rat was shaved from the rostral edge of the scrotum to the caudal abdomen. The area was then washed with Betadine three times.

Surgery began with a 2 cm ventral midline incision, just above the scrotal area. Through the incision, the vas deferens was located and externalized. A 0.25 cm section of each duct was removed with a micro-cautery knife. The muscle walls were sutured and the external incision was closed using sterilized staples that were removed ten days after the surgery. Each surgical procedure lasted 15-30 min.

Each rat was given a 1 mL injection of 0.9% warm saline and ketorolac subcutaneously and then placed into a post-operative Plexiglas cage, heated by an electric heating pad. Following observation of locomotion and urination, each rat was returned to its individual home cage and was monitored daily throughout the recovery period.

Experimental Procedures

General Social Defeat Stress Procedure

At the beginning of the 10-min social defeat session, the female rat paired with one resident rat was taken out of the cage and placed into an empty, identical cage. The

intruder rat was then placed into the cage of the resident (direct interaction). The session was then closely monitored for submissive, supine posture expressed by the intruder. Each time the intruder exhibited a supine posture with the resident physically contacting the intruder for 2 sec or more, one defeat was counted. After three defeats or a total of 5 min elapsed (whichever came first), the intruder was taken out of the resident's cage momentarily and quickly placed into a 10 x 10 x 15 cm double-walled protective wire mesh cage. The intruder (within the wire mesh cage) was then placed back into the resident's cage for the remainder of the 10-min session (indirect interaction). The two intruder rats in each housing pair were run in simultaneous defeat sessions in the cages of two resident males that were placed side by side. Each pair of cage-mates received the same treatment. Each social defeat session was run between 8:00a.m. and 10:00a.m., and was videotaped for further analysis.

Social Defeat Stress Regimen: Experiment 1a

Thirty-six naïve male LE rats were assigned to six experimental groups (Table 2-1). The control rats from Group 1 (n = 6) remained in their original cages throughout the experiment and were rapidly decapitated between 8:00a.m. and 10:00a.m. on the final experimental day. These rats were exposed to no chronic and no acute stress (NC/NA). The intruders from Group 2 (n = 6) were exposed to social defeat stress once every 24 h across six experimental days and were killed by rapid decapitation between 8:00a.m. and 10:00a.m., 24 h after their final social defeat session. These rats were exposed to chronic, but not acute stress (C/NA). The intruders from Group 3 (n = 6) were only exposed to social defeat stress on the final experimental day and were then immediately decapitated, 10 min after the start of the social defeat session (t = 10 min). These rats were exposed to no chronic, just acute stress (NC/A-10). The intruders from Group 4 (n = 6) were only

exposed to social defeat stress on the final experimental day and were rapidly decapitated 30 min after the start of the social defeat session (t = 30 min). These rats were exposed to no chronic, just acute stress (NC/A-30). The intruders from Group 5 (n = 6) were exposed to social defeat stress once every 24 h over the course of six experimental days and were killed by rapid decapitation 10 min after the start of the social defeat session on the sixth day (t = 10 min). These rats were exposed to chronic and acute stress (C/A-10). The intruders from Group 6 (n = 6) were exposed to social defeat stress once every 24 h over the course of six experimental days and were killed by rapid decapitation 30 min after the start of the social defeat session on the sixth day (t = 30 min). These rats were exposed to chronic and acute stress (C/A-30). Each intruder from the repeatedly stressed groups was placed in the cage of a different resident each day of his chronic stress routine.

Social Defeat Stress Regimen: Experiment 1b

Eighteen naïve male LE rats were assigned to three experimental groups (Table 2-2). The unhandled control rats from Group 1 (n = 6) remained in their original cages throughout the experiment and were killed by rapid decapitation between 8:00a.m. and 10:00a.m. on the final experimental day. These rats were exposed to no chronic and no acute stress (NC/NA). The intruders from Group 2 (n = 6) were exposed to social defeat stress once every 72 h across thirty-four experimental days and were rapidly decapitated between 8:00a.m. and 10:00a.m., 24 h after their final social defeat session. These rats were exposed to chronic, but not acute stress (C/NA). The intruders from Group 3 (n = 6) were exposed to social defeat stress once every 72 h across thirty-four experimental days and were killed by rapid decapitation 30 min after the start of the social defeat session on the final day (t = 30 min). These rats were exposed to chronic and acute stress

(C/A). All intruder rats from Experiment 1b were exposed to a total of twelve social defeat sessions. Each intruder from the repeatedly stressed groups was placed in the cage of a different resident each day of his chronic stress routine.

Social Defeat Stress and Porsolt Swim Test: Experiment 2

Twenty-four naïve male LE rats were assigned to three experimental groups (Table 2-3). The unhandled control rats from Group 1 (n = 8) remained in their original cages throughout the social defeat portion of the experiment. These rats were exposed to no chronic and no acute social defeat stress (NC/NA). The intruders from Group 2 (n = 8) were only exposed to social defeat stress on the final day of the social defeat portion of the experiment. These rats were exposed to no chronic, just acute stress (NC/A). The intruders from Group 3 (n = 8) were repeatedly exposed to social defeat stress over the course of five days, once every 24 h. These rats were exposed to chronic and acute stress (C/A). Each repeatedly stressed intruder was placed in the cage of a different resident each day of his chronic stress routine. All of the rats from each of the three groups were then exposed to the Porsolt swim test.

Twenty-four hours after the final social defeat session, each rat from Experiment 2 was individually removed from its home cage and placed into a plastic cylinder filled with approximately 25 cm of clean tap water at 24-27°C. The depth of water allowed each rat to reach the bottom of the cylinder with its tail, with enough head room that the rat was unable to escape from the tank. The water in each cylinder was changed between trials. Each rat was subjected, one at a time, to a 15-min swim session then carefully dried and returned to its home cage. Each Porsolt swim session was run between 8:00a.m. and 10:00a.m., and was videotaped for further analysis.

Behavioral Assays

Number of defeats, latency to first defeat, freezing behavior, and exploratory locomotion were scored from the recorded social defeat sessions. Freezing behavior was recorded whenever the intruder remained motionless for at least 2 sec, and is reported as percent time during the direct interaction between the intruder and the resident. Exploratory locomotion was recorded every time the intruder crossed with all four paws from one third of the cage into the adjacent third of the cage. Vertical lines were drawn on the video image of each resident's home cage and exploratory locomotion was scored from the videotapes. Exploratory locomotion is reported as lines crossed per minute of direct interaction time. Two trained observers scored the social defeat stress videos from Experiments 1a and 1b and inter-observer reliability was assessed.

Immobility was recorded during the Porsolt swim tests whenever the rat balanced on its tail, completely motionless, or exhibited only slight forepaw movement for a minimum of 2 sec. This behavior is reported as total time spent immobile during the swim test. Two trained observers scored the swim test videos from Experiment 2 and inter-observer reliability was assessed.

Histological Assays

Immediately after decapitation in experiments 1a and 1b, 6 mL of trunk blood from each rat was collected in polypropylene tubes on ice with 600 µl of Na_2EDTA at 20 µg/µL. The blood samples were then immediately centrifuged at 1000 rcf for 5 min and the plasma fraction frozen in 300 µl aliquots at –80°C. The brain from each intruder was quickly removed, frozen in 2-methylbutane at -40°C, and stored at -80°C for future analysis of molecular variables involved in HPA axis function. The thymus and adrenal glands were removed from each rat and frozen separately at –80°C. These glands were

later weighed to determine the health and stress status of each rat at time of death. Plasma ACTH and CORT concentrations from the rats were later analyzed with radioimmunoassay (RIA). ACTH RIAs were run with kits from Alpco Diagnostics (Salem, NH) and CORT RIAs were run with kits from Diagnostic Products Corporation (Los Angeles, CA).

Statistical Analyses

Potential between-groups differences in number of defeats and latency to first defeat were analyzed using two one-way analyses of variance (ANOVAs) to compare the five groups of intruders (i.e. three repeatedly stressed, and two just acutely stressed) in Experiment 1a during their first exposure to social defeat stress. A 3x6 (group x session) repeated-measures ANOVA (for Experiment 1a) and a 2x12 (group x session) repeated-measures ANOVA (for Experiment 1b) were used to examine potential differences in number of defeats between groups and across experimental sessions for the repeatedly stressed groups. A 3x6 (group x session) repeated-measures ANOVA (for Experiment 1a) and a 2x12 (group x session) repeated-measures ANOVA (for Experiment 1b) were used to examine potential differences in latency to first defeat between groups and across experimental sessions for the repeatedly stressed groups.

Potential between-groups differences in freezing responses were analyzed using a one-way ANOVA to compare freezing behavior between the five groups in Experiment 1a during their first exposure to social defeat stress. A 3x6 (group x session) repeated-measures ANOVA (for Experiment 1a) and a 2x12 (group x session) repeated-measures ANOVA (for Experiment 1b) were used to examine potential freezing behavior differences between groups and across experimental sessions for the repeatedly stressed groups.

Potential between-groups differences in exploratory locomotion were analyzed using a one-way analysis of variance (ANOVA) to compare exploratory locomotion between the five groups in Experiment 1a during their first exposure to social defeat stress. A 3x6 (group x session) repeated-measures ANOVA (for Experiment 1a) and a 2x12 (group x session) repeated-measures ANOVA (for Experiment 1b) were used to examine potential exploratory locomotion differences between groups and across experimental sessions for the repeatedly stressed groups.

Potential between-groups differences in plasma ACTH concentrations and in plasma CORT concentrations were analyzed with one-way ANOVAs in each of Experiments 1a and 1b. Also, potential between-groups differences in adrenal weights and in thymus weights were analyzed with one-way ANOVAs in each of Experiments 1a and 1b. The results were further analyzed using pre-planned Newman-Keuls multiple comparison tests for all significant ANOVAs.

Potential between-groups differences in immobility duration scores for the 15-min Porsolt swim test session in Experiment 2 were analyzed using a one-way ANOVA. The results were further analyzed using Newman-Keuls multiple comparison tests. The data were then analyzed in 5-min bins using a 3x3 (group x bin) repeated-measures ANOVA. The results were further analyzed using Newman-Keuls multiple comparison tests, making pair-wise comparisons between each stressed group and the control group, for each bin.

Inter-observer agreement was assessed for freezing behavior in Experiments 1a and 1b by comparing total times recorded by each trained observer for each social defeat session. Inter-observer agreement was also assessed for exploratory locomotion in

Experiments 1a and 1b by comparing total number of line crossings recorded by each trained observer for each social defeat session. Inter-observer agreement was assessed for immobility duration scores for the Porsolt swim test from Experiment 2 by comparing total immobility times recorded by each trained observer for each test session.

Table 2-1. Experiment 1a, group assignments

Group	**Repeated Stress**	**Acute Stress**	**Kill Time**
NC/NA			
C/NA	X		t = 24 h
NC/A-10		X	t = 10 min
NC/A-30		X	t = 30 min
C/A-10	X	X	t = 10 min
C/A-30	X	X	t = 30 min

Table 2-2. Experiment 1b, group assignments

Group	**Repeated Stress**	**Acute Stress**	**Kill Time**
NC/NA			
C/NA	X		t = 24 h
C/A	X	X	t = 30 min

Table 2-3. Experiment 2, group assignments

Group	**Repeated Stress**	**Acute Stress**	**Porsolt Swim Test**
NC/NA			X
NC/A		X	X
C/A	X	X	X

CHAPTER 3
RESULTS

Experiment 1a: Six Daily Sessions of Social Defeat Stress

During their first exposure to social defeat stress, the rats from all five of the groups in Experiment 1a did not show statistically different numbers of defeats ($F(4, 25) = 0.2419$, $p < 0.9118$; Fig. 3-1A) nor latencies to first defeat ($F(4, 25) = 0.7308$, $p < 0.5795$; Fig. 3-1B). Each of the three repeatedly stressed groups (C/NA, C/A-10, and C/A-30), showed no significant between-groups difference, time effect, or group by time interaction effect in number of defeats ($F(2, 15) = 1.603$, $p < 0.2340$; $F(5, 15) = 0.3463$, $p < 0.8831$; $F(10, 15) = 0.3139$, $p < 0.9753$; Fig. 3-1A) nor latencies to first defeat across the six experimental sessions ($F(2, 15) = 1.571$, $p < 0.2401$; $F(5, 15) = 0.2106$, $p < 0.9570$; $F(10, 15) = 0.5255$, $p < 0.8669$; Fig. 3-1B).

During their first exposure to social defeat stress, the rats from all five of the groups did not show statistically different amounts of freezing behavior ($F(4, 25) = 0.1757$, $p < 0.8405$; Fig. 3-2A). Each of the three repeatedly stressed groups showed significantly increased freezing behavior across the six experimental sessions ($F(5, 75) = 17.08$, $p < 0.0001$; Fig. 3-2A), reaching asymptote by day 3, with no significant between-groups differences or group by time interaction effect.

During their first exposure to social defeat stress, the rats from all five groups did not show statistically different amounts of exploratory locomotion ($F(4, 25) = 0.5711$, $p < 0.5767$; Fig. 3-2B). Each of the three repeatedly stressed groups showed significantly decreased exploratory locomotion across the six experimental sessions ($F(5, 75) = 11.76$,

$p < 0.0001$; Fig. 3-2B), reaching asymptote by day 4, with no significant between-groups differences or group by time interaction effect.

Exposure to acute social defeat (NC/A and C/A) produced significant elevations in circulating ACTH concentrations when compared with the ACTH concentrations in the control rats (NC/NA) ($F (5, 30) = 3.874$, $p < 0.0079$; Fig. 3-3A). Circulating ACTH concentrations were not elevated in the chronically, but not acutely, stressed rats (C/NA). Exposure to social defeat stress for all groups (including C/NA) produced significant elevations in circulating CORT concentrations when compared with the CORT concentrations in the control rats ($F (5, 30) = 20.79$, $p < 0.0001$; Fig. 3-3B).

Exposure to social defeat significantly decreased thymus masses in Groups C/A-10 and C/A-30, but not for the other groups ($F (2, 15) = 5.651$, $p < 0.0148$; Fig. 3-4A). There were no significant differences in adrenal masses between the rats in the stressed groups and the rats in the control group ($F (5, 30) = 0.9299$, $p < 0.4756$; Fig. 3-4B).

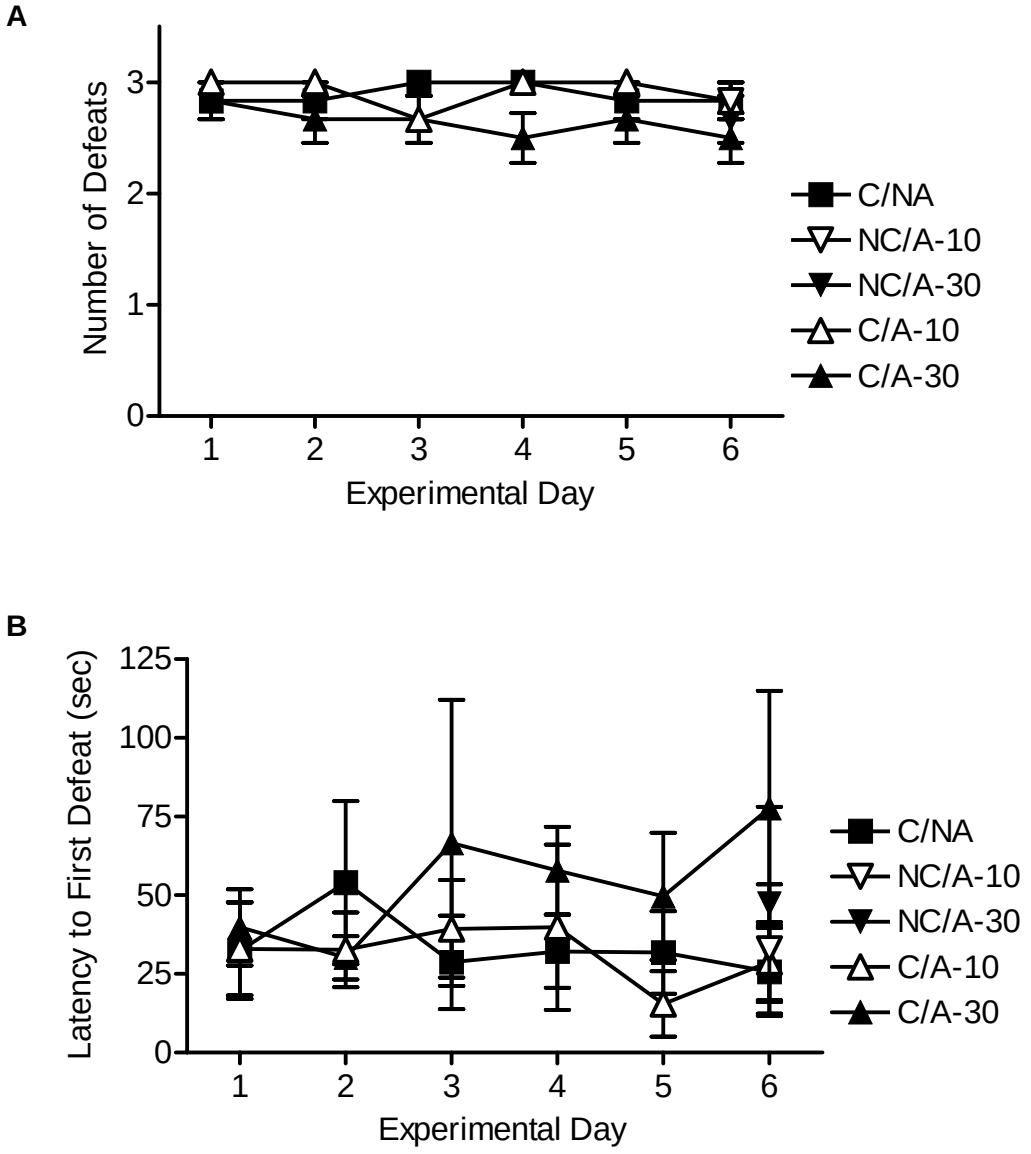

Figure 3-1. Social defeats per daily session. The rats exposed to repeated social defeat stress (C/NA, C/A-10, C/A-30) exhibited equivalent (A) number of defeats per session and (B) latency to first defeat per session across the 6 experimental sessions. The rats that received only one acute defeat session (NCA-10 and NCA-30) were stressed at the same time as the final stress session for the chronically stressed groups, and so the values for these groups are illustrated on day 6. Values expressed are group means ± SEM (n = 6 rats per group). Abbreviations: C/NA = chronic stress/no acute stress, NC/A = no chronic stress/acute stress, C/A = chronic stress/acute stress.

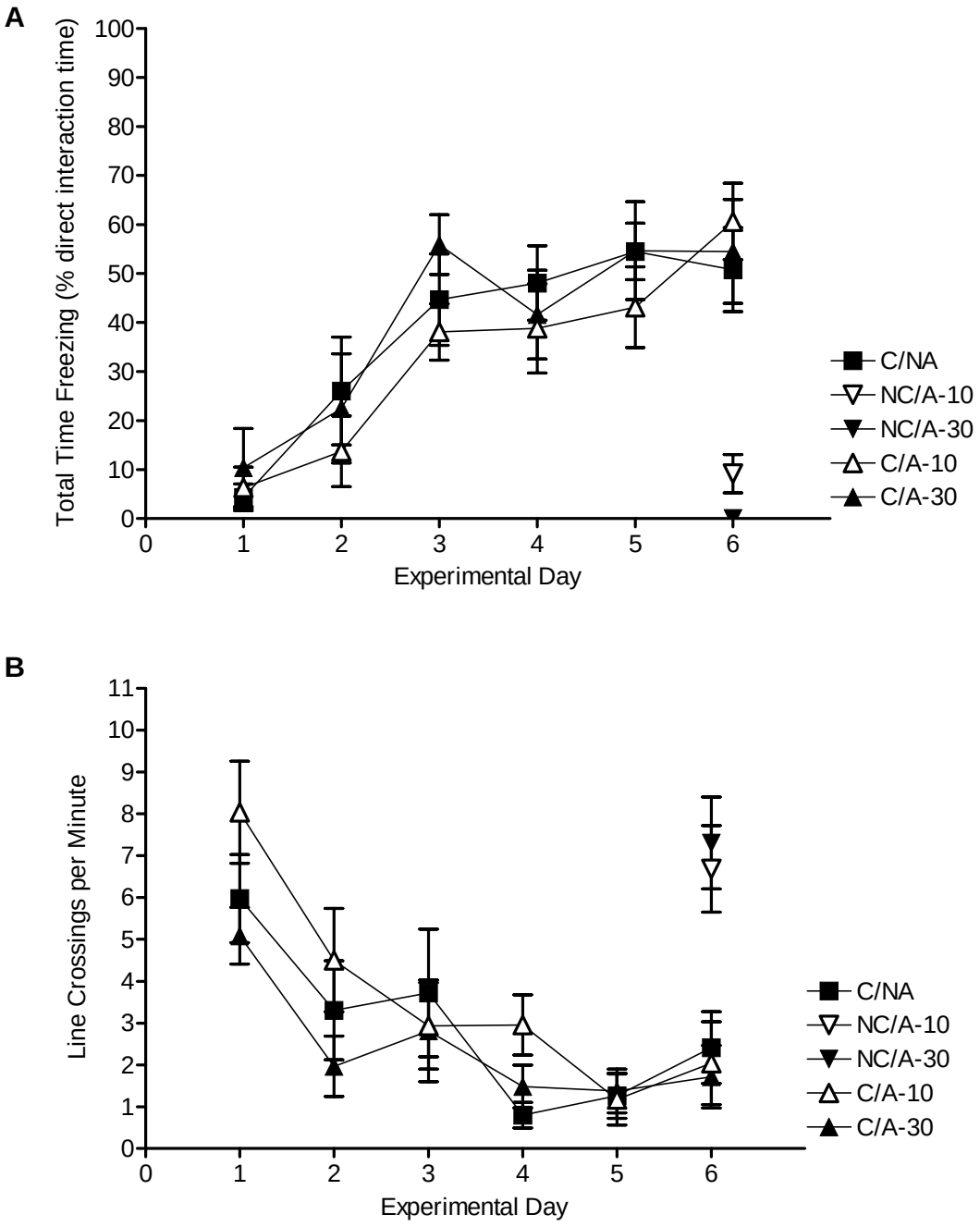

Figure 3-2. Intruder behavior during daily social defeat sessions. The rats exposed to repeated social defeat stress exhibited (A) increases in freezing behavior and (B) decreases in exploratory locomotion across the 6 experimental sessions. The rats that received only one acute defeat session were stressed at the same time as the final stress session for the chronically stressed groups, and so the values for these groups are illustrated on day 6. Values expressed are group means ± SEM (n = 6 rats per group).

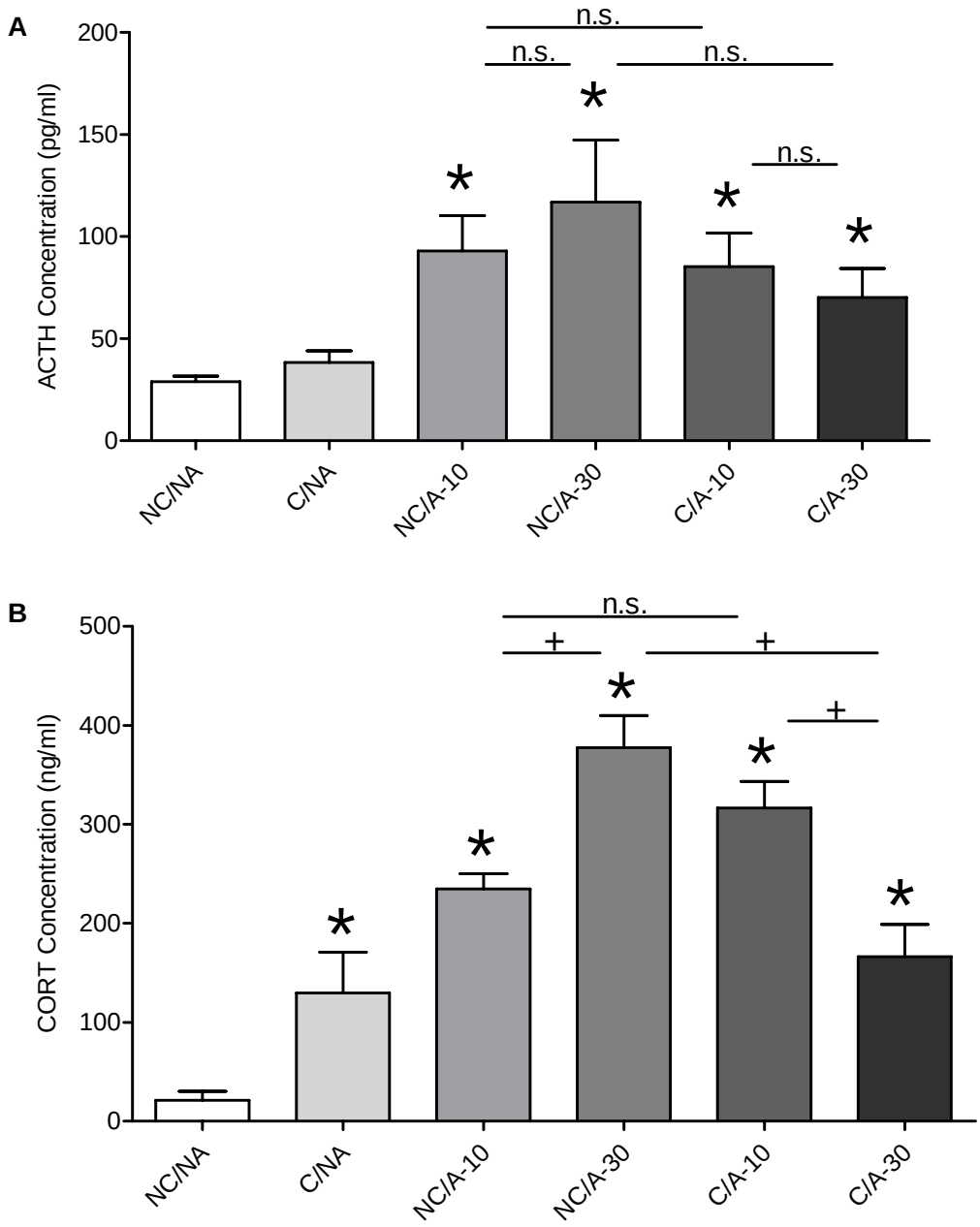

Figure 3-3. Circulating hormones after daily social defeat stress. The rats exposed to social defeat stress had elevated circulating concentrations of (A) ACTH and (B) CORT when compared to basal concentrations in control rats. Values expressed are group means ± SEM (n = 6 rats per group). Significant differences between the socially defeated rats and the unhandled control rats (NC/NA) are expressed as [*] $p < 0.05$. Significant differences in pre-planned comparisons between the stressed groups of rats are expressed as [+] $p < 0.05$, with lines connecting the groups that were compared. Additional abbreviation: NC/NA = no chronic stress/no acute stress.

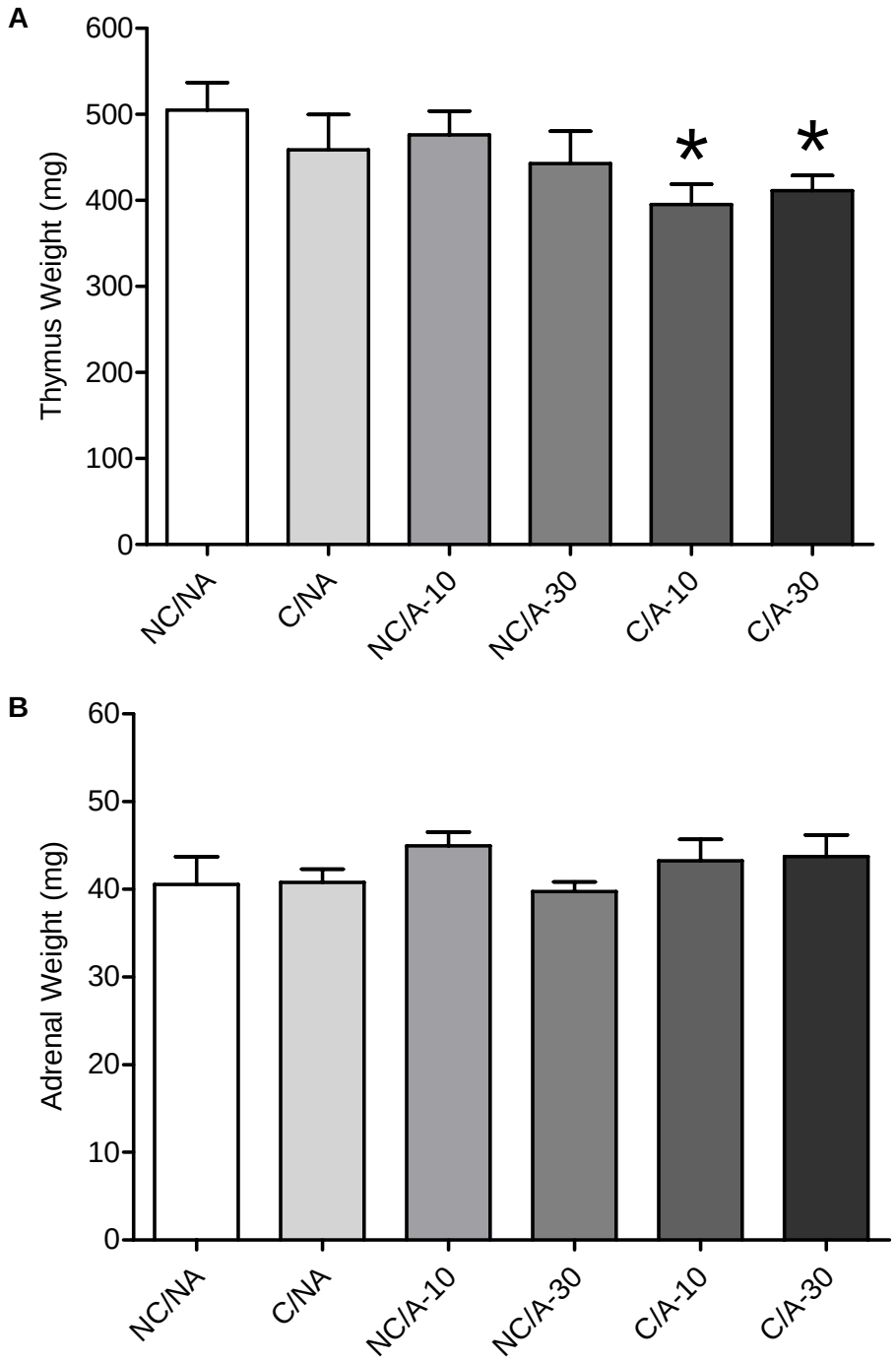

Figure 3-4. Effects of daily social defeat stress on glandular masses. Social defeat stress (A) significantly decreased thymus masses for 2 of the repeatedly stressed groups (C/A-10 and C/A-30), but (B) did not significantly affect adrenal gland masses for any group. Values expressed are group means ± SEM (n = 6 rats per group). Significant differences between the socially defeated rats and the control rats are expressed as [*] $p < 0.05$.

Experiment 1b: Twelve Intermittent Sessions of Social Defeat Stress

There were no significant between-groups differences, time effect, or group by time interaction effect in number of defeats ($F(1, 10) = 0.0787$, $p < 0.7847$; $F(11, 10) = 0.9032$, $p < 0.5399$; $F(11, 10) = 0.8203$, $p < 0.6199$; Fig. 3-5A) nor latency to first defeat ($F(1, 10) = 0.4653$, $p < 0.5107$; $F(11, 10) = 0.8400$, $p < 0.6007$; $F(11, 10) = 0.3414$, $p < 0.9743$; Fig. 3-5B) for the repeatedly stressed groups (C/NA and C/A) in Experiment 1b.

Freezing behavior significantly increased for both of the repeatedly stressed groups across the twelve experimental sessions ($F(11, 10) = 6.224$, $p < 0.0001$; Fig. 3-6A), with no significant between-groups differences or group by time interaction effect.

Exploratory locomotion significantly decreased for both of the repeatedly stressed groups across the twelve experimental sessions ($F(11, 10) = 7.252$, $p < 0.0001$; Fig. 3-6B), with no significant between-groups differences or group by time interaction effect.

Acute exposure to social defeat stress produced significant elevations in circulating ACTH concentrations when compared with the basal ACTH concentrations in the control rats ($F(2, 15) = 4.525$, $p < 0.0290$; Fig. 3-7A). Acute exposure to social defeat stress also produced significant elevations in circulating CORT concentrations when compared with the basal CORT concentrations in the control rats ($F(2, 15) = 5.853$, $p < 0.0132$; Fig. 3-7B). These elevations in circulating hormones were limited to the rats that were stressed acutely before termination (C/A) – the basal concentrations in the other chronically stressed rats (C/NA) did not significantly differ from the basal concentrations in the control rats (NC/NA).

There were no significant differences in thymus masses ($F(2, 15) = 0.04457$, $p < 0.9565$; Fig. 3-8A) or adrenal masses ($F(2, 15) = 1.826$, $p < 0.1951$; Fig. 3-8B) between the rats in the socially defeated groups and the rats in the control group.

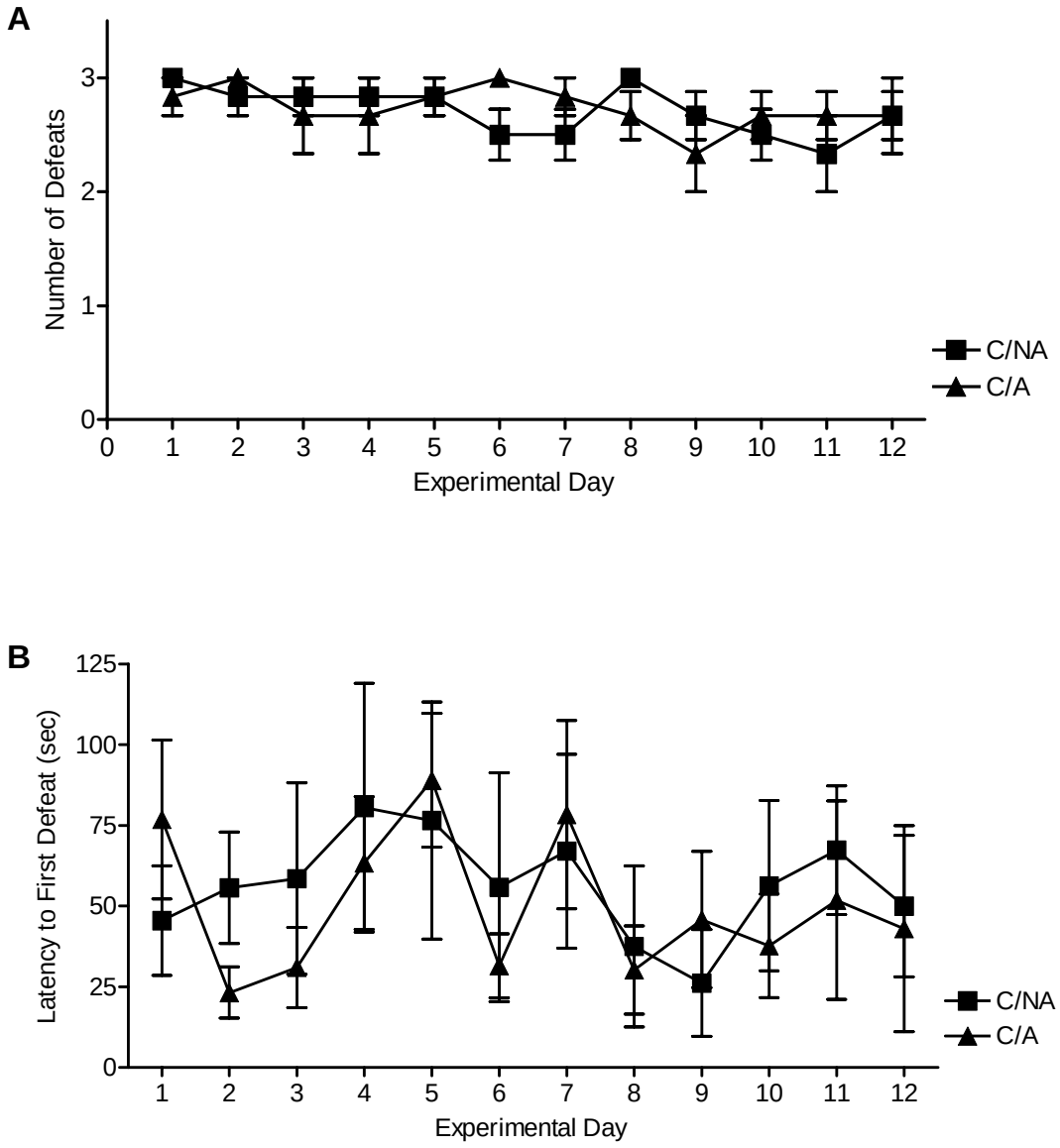

Figure 3-5. Social defeats per intermittent session. The rats exposed to repeated social defeat stress (C/NA and C/A) exhibited equivalent (A) number of defeats per session and (B) latency to first defeat per session across the 12 experimental sessions. Values expressed are group means ± SEM (n = 6 rats per group).

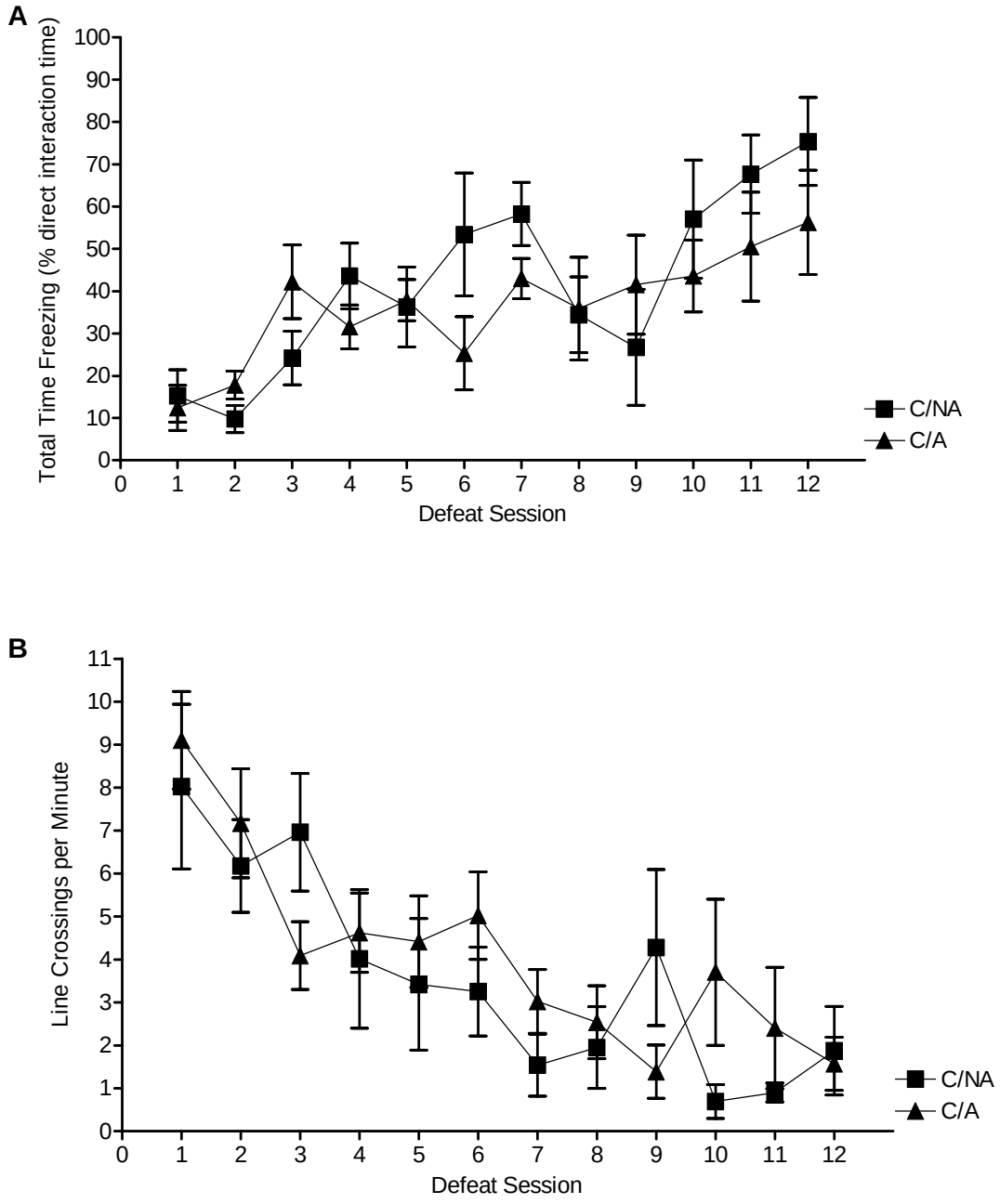

Figure 3-6. Intruder behaviors during intermittent social defeat sessions. The rats exposed to repeated social defeat stress exhibited (**A**) increases in freezing behavior and (**B**) decreases in exploratory locomotion across 12 experimental sessions. Values expressed are group means ± SEM (n = 6 rats per group).

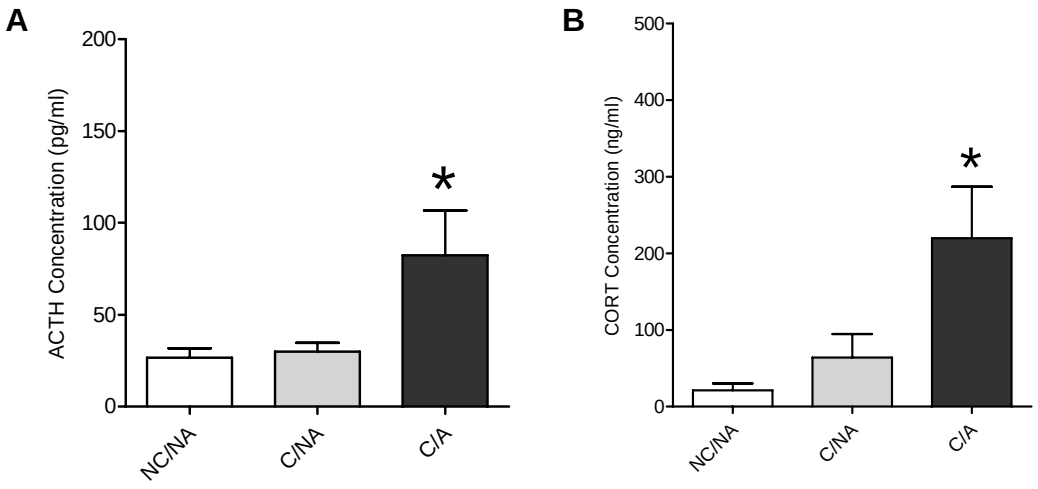

Figure 3-7. Circulating hormones after intermittent social defeat stress. Immediately after, but not 24 h later, the rats exposed to repeated social defeat stress had elevated circulating concentrations of (A) ACTH and (B) CORT when compared to basal concentrations in the control rats (NC/NA). Values expressed are group means ± SEM (n = 6 rats per group). Significant differences between the socially defeated rats and the unhandled control rats are expressed as [*] $p < 0.05$.

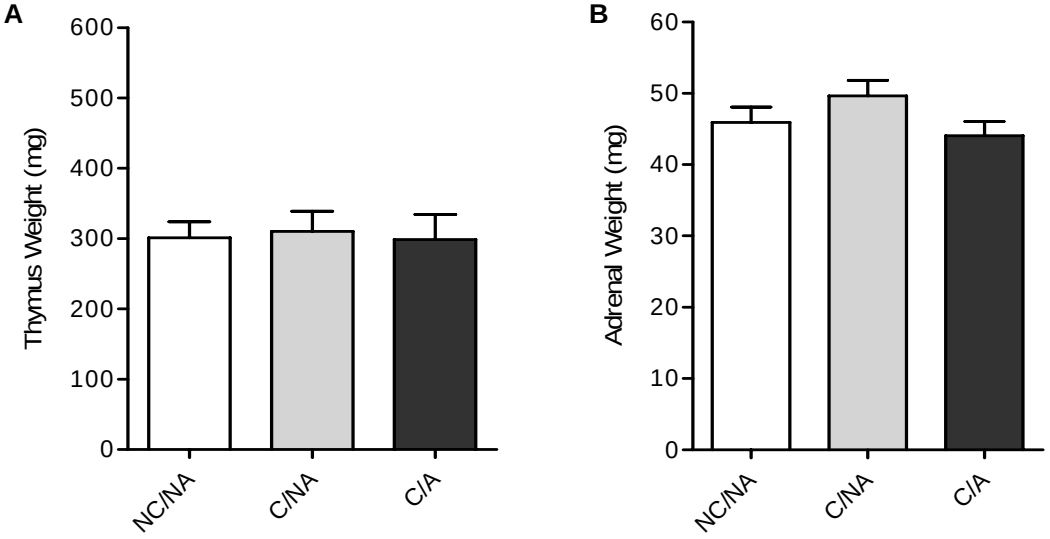

Figure 3-8. Effects of intermittent social defeat stress on glandular masses. Repeated social defeat stress had no effect on (A) thymus masses or (B) adrenal gland masses. Values expressed are group means ± SEM (n = 6 rats per group).

Experiment 2: Social Defeat Stress Followed by Porsolt Swim Testing

Exposure to chronic social defeat stress (C/A) produced a significant increase in total immobility time during the 15-min forced swim when compared with the immobility times for the control rats that were not exposed to social defeat stress (NC/NA) ($F(2, 21) = 5.363$, $p < 0.0131$; Fig. 3-9). When the data were analyzed in 5-min bins, there was a significant between-groups effect as well as a significant time effect ($F(2, 42) = 2.424$, $p < 0.0150$; $F(2, 42) = 72.74$, $p < 0.0001$; Fig. 3-10), but no significant group by time interaction effect ($F(4, 42) = 1.270$, $p < 0.2970$; Fig. 3-10).

Inter-Observer Reliability

The two observer's recordings of total time freezing for the social defeat sessions from Experiments 1a and 1b combined differed by less than 20 sec for 94% of the sessions and never differed by more than 28 sec. The two observer's recordings of exploratory locomotion during social defeat sessions were identical in 70% of the sessions and never differed by more than 3 lines crossed.

The two observer's recordings of total immobility time during the Porsolt swim test differed by less than 40 sec in 92% of the sessions and never differed by more than 43 sec.

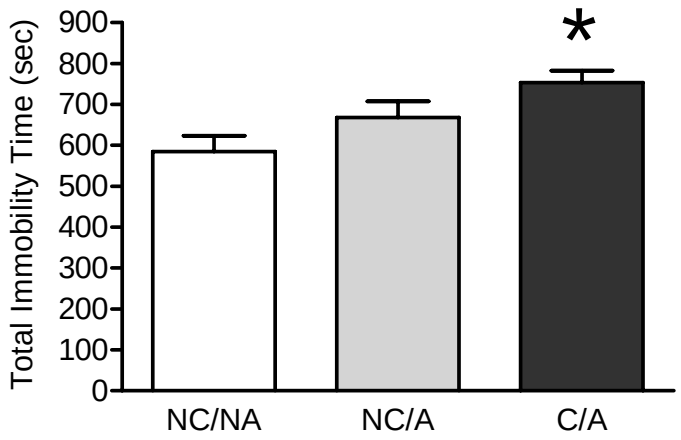

Figure 3-9. Immobility during the Porsolt swim test. The rats exposed to repeated social defeats stress (Chronic) exhibited increased immobility for the duration of the Porsolt forced swim test, when compared to the immobility times for the intruder rats exposed to acute social defeat stress (Acute) and the control rats (No SD). Values expressed are group means ± SEM (n = 8 rats per group). Significant differences between the socially defeated rats and the unhandled control rats are expressed as [*] $p < 0.05$. Additional abbreviation: No SD = no social defeat stress.

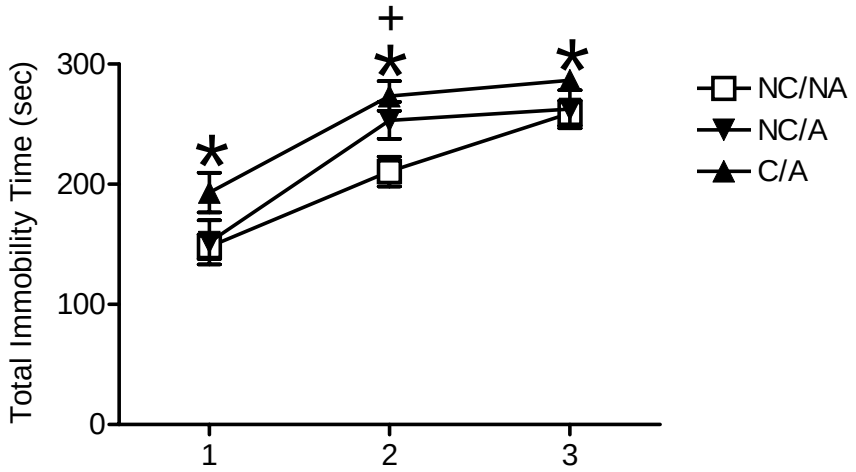

Figure 3-10. Immobility measured in 5-minute bins during the Porsolt swim test. There were significant main effects for time and between-groups difference when comparing the socially defeated groups (Acute and Chronic) to the control group (No SD). Values expressed are group means ± SEM (n = 8 rats per group). Significant differences between the chronically defeated rats and the controls are expressed as [*] $p < 0.05$. Significant differences between the acutely defeated rats and the controls are expressed as [+] $p < 0.05$.

CHAPTER 4
DISCUSSION

Overall, repeated social defeat stress produced behavioral, hormonal, and glandular changes that model some of the symptoms that are seen in depressed humans. Behavioral despair, HPA axis dysregulation, and thymus involution were found in rats that were exposed to daily social stress; and these effects were greater overall than the effects that were seen in the rats that were stressed intermittently.

Daily Social Defeat Stress

The equivalent mean freezing scores and the equivalent mean locomotion scores during the first social defeat session indicate that all five groups that experienced social defeat stress were comparable at the beginning of the experiment. The equivalent progressive increases in freezing behavior and the equivalent decreases in exploratory locomotion for the three repeatedly stressed groups indicate that these groups underwent comparable stress-induced behavioral changes across social defeat sessions. These behaviors, however, did not diminish the persistent displays of dominant behavior (consistent number of defeats and equivalent latencies to first defeat) by the resident rats. After 3-4 days of stress exposure, the behavioral curves for both freezing behavior and exploratory locomotion reached asymptote, suggesting that the behavioral changes associated with daily social defeat stress do not progress beyond the first few exposures.

The equivalent mean ACTH concentrations and the equivalent mean CORT concentrations 10 min after the onset of stress between the groups with and without a history of stress suggest that there is similar activation of the HPA axis for these groups.

However, the lower CORT concentrations 30 min after the onset of stress in the repeatedly stressed group compared to the CORT concentrations in the acutely stressed group suggests a sensitization in the negative feedback regulation of CORT.

Decreased thymus mass and/or increased adrenal mass has been shown to result from chronic stress regimens involving physical and/or psychological stressors (for examples, see Blanchard et al., 1998; Bryant et al., 1991; Simpkiss and Devine, 2003) as well as from major depression in victims of suicide (Szigethy et al., 1994). Given that two of the three groups that were stressed daily (C/A-10 and C/A-30) exhibited thymus involution while one (C/NA) did not, it cannot be concluded that the daily stress regimen consistently caused a decrease in thymus mass. These findings, along with adrenal mass equivalency across groups regardless of number of stress exposures, suggest that the chronic social defeat stress regimen of six daily exposures may not have been adequate to consistently alter glandular masses. A longer or unpredictable social defeat stress regimen could possibly generate consistent thymus involution and adrenal hypertrophy. Perhaps an additional stressor for the intruders, such as isolation housing to ensure more timid behavior (Kabbaj et al., 2000), could contribute to the effectiveness of the regimen. Also, using a restraint stress harness instead of the protective wire mesh cage could elevate the efficiency of the regimen by producing an inescapable condition for the intruders.

Intermittent Social Defeat Stress

The equivalent progressive increases in freezing behavior and the equivalent progressive decreases in exploratory locomotion in the intermittent stress regimen indicate that the repeatedly stressed groups underwent comparable stress-induced behavioral changes. However, the escalation of freezing behavior and the decline of

exploratory locomotion occurred more gradually when the social defeat sessions were 72 hours apart than when the social defeat sessions occurred daily. This suggests that the intermittent stress regimen was not as efficient at inducing these behavioral changes, although the overall magnitudes of the behavioral effects were eventually roughly equivalent. Once again, the intruders' behavioral adaptations did not decrease the displays of agonistic behavior from the residents (consistent number of defeats and equivalent latencies to first defeat across groups and days).

The equivalence in basal CORT concentrations as well as the equivalence in glandular masses between the chronically stressed animals and the control animals indicates that the temporally spaced regimen was not potent enough to sufficiently alter hormonal or glandular basal states. Even though the more temporally spaced chronic stress regimen was able to produce equivalent effects with regards to behavioral changes (albeit, more slowly), it was less effective than the daily regimen in producing the hormonal and glandular changes that correlate with some of the symptoms of major depression. The results indicate, therefore, that there may be a critical window for vulnerability to an additional stressor—it is possible that the rats were able to partially recover from the initial stressor by the next exposure in the more temporally spaced chronic regimen.

Relevance to Previous Work

Overall, these results confirm and extend a previous report that daily social defeat is an effective emotional stressor for male rats. In a study by Haller and colleagues (1999), intruder rats were exposed to resident rats for 4 hours on four consecutive days, producing increased basal CORT concentrations in the intruders. The daily social defeat stress regimen used in our study effectively increased basal CORT concentrations as

well, with briefer stressful interactions than previously reported. However, in the same study, Haller and colleagues found increased adrenal mass with no decrease in thymus mass, results opposite to those seen after our similar daily chronic stress regimen. Perhaps it is necessary for a daily chronic stress regimen to extend past four (or six) days to generate reliable glandular changes.

The HPA axis response to an acute stressor largely depends upon whether the acute stressor is heterotypic or homotypic. In a study by Armario and colleagues (2004), investigators reported that when previously-stressed rats (via immobilization) were presented with a heterotypic stressor (forced swimming), a minor sensitization was observed. Also, following exposure to a severe stressor (such as shock, restraint, immobilization, or large doses of endotoxin), there was habituation of the HPA axis response to a homotypic stressor. These results differ from the results obtained after daily social defeat stress. Following our repeated daily social defeat stress regimen, there was a robust activation but rapid shutdown of CORT release. Most probably, the difference is due to the fact that Armario and colleagues utilized a single stressor followed by either homotypic or heterotypic challenges. Our model of social defeat does not appear to function as a homotypic stress regimen. There are several variables involved in the direct interaction phase of social defeat stress (physical contact, olfactory cues, and ultra-sonic vocalizations) as well as the introduction of a different resident rat with each exposure. Conceivably, the use of a different resident for each stress exposure and the potentially different threats imposed by these different residents creates a novel, therefore heterotypic, situation with each session.

In general, the behavioral changes in the intruder rats after repeated social defeat stress model some of the symptoms expressed in clinically depressed patients, including impairment in social or occupational functioning and loss of interest (DSM IV, 1994). Also, the elevated basal CORT concentrations after daily social defeat stress during the nadir of the daily cycle confirm previous findings from our laboratory (Lopes and Devine, 2004) and mimic the augmented circulating hormones found in clinically depressed patients (Linkowski et al., 1985; Pfohl et al., 1985).

Porsolt Swim Test

Based on the combined results from the daily and the intermittent stress regimens, a daily social defeat stress regimen was used to for the Porsolt study to optimize the potential to produce behavioral despair. The fact that acute social defeat stress did not produce as much immobility as repeated stress indicates that the neuronal plasticity caused by repeated exposure to social stress (demonstrated through hormonal and behavioral plasticity) is a determining factor in the expression of behavioral despair.

A 5-min swim test is commonly used for detection of behavioral despair (Gavioli et al., 2003; Hinojosa et al., 2006; Porsolt et al., 1978; Rygula et al., 2005). Interestingly, when analyzed in 5-min bins, the data suggest that in all the bins the repeatedly stressed rats exhibited more immobility than the controls did, and in one bin (5-10 min) the acutely defeated rats also exhibited more immobility than the controls did. Therefore, a 10-min session may be necessary to closely examine the subtle differences between groups that have a less severe stress history and non-stressed control rats.

Overall, these results model the behavioral despair, or low mood and anhedonia, found in clinically depressed patients (for review, see Harrison, 2002). Also, the significantly elevated immobility times for the repeatedly stressed rats confirm and

extend the results from a previous report. In a study by Rygula and colleagues (2005), investigators used a more extensive stress regimen of hour-long daily sessions of social defeat for five straight weeks to elicit behavioral despair in subsequent Porsolt swim testing. The daily social defeat stress regimen used in our study effectively increased immobility in the repeatedly stressed rats as well, with briefer stressful interactions and a shorter regimen than previously reported.

Conclusions and Future Directions

In conclusion, the behavioral, hormonal, and glandular changes produced by repeated social defeat closely resemble many of the psychopathological symptoms in patients with major depression. The daily social defeat stress regimen provides an interesting and effective model for stress-induced psychopathology.

The effect of repeated social defeat on the overall circadian rhythm was not evaluated, nor was the persistence of the altered regulation across days, weeks, or months. Both of these topics may be interesting issues for future studies. Also, potential adaptations in stress-related molecules (CRH and AVP in the hypothalamus, CRH1 and V1b in the pituitary and amygdala, and mineralocorticoid receptor and gluccocorticoid receptor in the hippocampus) after social defeat should be investigated. These studies will enhance our growing knowledge of the neurobiological basis for stress-induced psychopathology (e.g., major depression).

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BIOGRAPHICAL SKETCH

Kristen L. Stone graduated in May 2004 from the University of Central Florida (Orlando) with her Bachelor of Science degree in psychology. She began her graduate education in the Psychology Department at the University of Florida (Gainesville) in August 2004, working toward her Master of Science degree, in the Behavioral Neuroscience program.