EFFECTS OF AMYGDALA LESIONS ON BEHAVIORAL AND NEUROENDOCRINE RESPONSES TO DEFEAT IN MALE SYRIAN HAMSTERS

by

JUDITH ALICIA ASKEW DUNCAN

(Under the direction of Gaylen L. Edwards, Ph.D.)

ABSTRACT

In hamsters, territorial aggression normally directed toward a non-aggressive intruder is replaced by submissive/defensive behaviors in hamsters that have previously experienced social defeat. This phenomenon has been labeled conditioned defeat. The present study investigated the role of the amygdala in the modulation of behavioral and neuroendocrine responses to defeat. In Experiments 1 and 2, male Syrian hamsters received either bilateral electrolytic or sham lesions of the central/basolateral (CE/BLA) nuclei of the amygdala. In Experiment 1, agonistic behavior was recorded during a 10-min social encounter with an aggressive male resident. Bilateral amygdala lesions had no effect on behavioral and neuroendocrine responses of the hamsters during an encounter with an aggressive resident. In Experiment 2, subjects were placed in an aggressive resident's home cage for 10-min with either the resident present or absent. As in Experiment 1, agonistic behavior was recorded during social encounters with aggressive male residents. Amygdala lesions had no effect on behavioral responses to an aggressive resident during the initial defeat. Twenty-four hours later, defeated and non-defeated hamsters were exposed to a non-aggressive intruder for 10 min. Lesioned, non-defeated hamsters exposed to a non-aggressive opponent exhibited increases in non-social and decreases in social behavior but exhibited similar levels of aggression and submission. By contrast, lesioned hamsters that had previously experienced defeat exhibited significantly less submissive behavior during testing. Plasma levels of ACTH and cortisol were similar for all subjects. While the effects of CE/BLA lesions on behavior appear to depend upon the social history of the subject, these lesions do not block the ability of subjects to produce normal submissive responses to an aggressive opponent. In addition, it appears that the CE/BLA amygdala region is not critical in the modulation of neuroendocrine responses of defeat stress. Data suggest that the CE/BLA is involved in the behavioral plasticity observed following an acute social defeat in Syrian hamsters.

INDEX WORDS: ACTH, Agonistic behavior, Amygdala, Conditioned defeat, Cortisol, Limbic system, Neuroendocrine, Social defeat, Social conflict, Stress

EFFECTS OF AMYGDALA LESIONS ON BEHAVIORAL AND NEUROENDOCRINE RESPONSES TO DEFEAT IN MALE SYRIAN HAMSTERS

by

JUDITH ALICIA ASKEW DUNCAN

B. S., Presbyterian College, 1992

M. S., The University of Georgia, 1998

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2002

EFFECTS OF AMYGDALA LESIONS ON BEHAVIORAL AND NEUROENDOCRINE RESPONSES TO DEFEAT IN MALE SYRIAN HAMSTERS

by

JUDITH ALICIA ASKEW DUNCAN

Approved:

Major Professor: Gaylen L. Edwards

Committee: Bradford N. Bunnell
Kim L. Huhman
Philip V. Holmes
Roger K. Thomas
Rod K. Dishman

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2002

DEDICATION

This dissertation is dedicated to my son, Matthew, for whom my love never-ending. Your laughter and your smiles have been meant so much to me. I love you with all my heart.

ACKNOWLEDGEMENTS

I would like to acknowledge those individuals who played an important role in the completion of this dissertation. I especially would like to thank my parents, Ollie Askew and Emily Knighton, for their love and support. I would like to thank Dr. Meyerhoff, Dr. Saviolakis and their staff at Walter Reed for performing the radioimmunoassays. I thank the staff of Animal Resources at Georgia State University for their expert animal care. I thank Dr. Bunnell for his support in this project and throughout my graduate career. Many thanks to Dr. Stahl, Dr. Gonzalez, Dr. Jasnow, Jeris Israel, and Matia Banks for their assistance and advice. I would like to thank Alicia Faruzzi for not only her assistance and advice, but also her support. I am also tremendously grateful for Dr. Huhman's guidance and assistance, which were crucial in completion of this project.

TABLE OF CONTENTS

Page

INTRODUCTION

Most mammals experience social conflict during some part of their lives. In comparison to stress models that use artificial stressors such as shock and immobilization, animal models that use social conflict to induce defeat appear more representative of stressful situations that individuals actually encounter on a daily basis. Social conflict thus provides a method to investigate the effects of biologically relevant stressors as well as the neurobiological mechanisms subserving stress-induced changes in physiology and behavior.

Social defeat has been utilized in many studies investigating neuroendocrine and behavioral stress responses (Hebert, Lumley, & Meyerhoff, 1998; Huhman, Bunnell, Mougey, & Meyerhoff, 1990; Huhman, Moore, Ferris, Mougey, & Meyerhoff, 1991; Huhman, Moore, Mougey & Meyerhoff, 1992; Martinez, Calvo-Torrent & Pico-Alfonso, 1998; Politch & Leshner, 1977). In hamsters, a conspecific, dyadic encounter leads to the rapid establishment of a dominant-subordinate relationship (Huhman et al., 1991). The defeated animal shows a variety of submissive postures and increased hypothalamic-pituitary-adrenal (HPA) activity (Huhman et al., 1990). Similar studies indicate that defeat experiences may alter behavioral responses to future social experiences (Hebert, Potegal, Moore, Evenson, & Meyerhoff, 1996; Potegal, Huhman, Moore, & Meyerhoff, 1993). Potegal et al. (1993) report a profound behavioral change in previously-defeated male golden hamsters. Subsequent to defeat by a larger and more aggressive hamster, normal aggressive behavior of a resident hamster toward a non-aggressive intruder is replaced by defensive-submissive behaviors. This behavioral change has been called "conditioned defeat" (Potegal et al., 1993).

One area of interest with respect to social conflict and social defeat has been to investigate the neuroanatomical substrates of agonistic behavior. Agonistic behavior encompasses not only aggressive behaviors, including threat or attack, but also submissive-defensive behaviors, including flight/escape as well as other more passive behaviors that serve to inhibit aggression from conspecifics. Aggression can be further divided into two classifications, offensive or defensive aggression (Blanchard & Blanchard, 1988 for review).

Offensive and defensive aggression appear to be regulated by different neural sites (Albert & Walsh, 1984; Blanchard & Blanchard, 1988). Data indicate that the amygdala, medial preoptic area (MPOA), anterior hypothalamus (AH), ventromedial hypothalamus (VMH), septum, periaqueductal gray (PAG), and bed nucleus of the stria terminalis (BNST) are neuroanatomical substrates of defensive aggression (Blanchard & Blanchard, 1988 for review) and that the dorsomedial tegmentum, substantia nigra, and ventromedial tegmentum are neuroanatomical substrates of offensive aggression (Adams, 1987; Albert & Walsh, 1984). In the hamster, Delville, De Vries, & Ferris (2000) report that c-*fos* immunoreactivity in the medial amygdaloid nucleus (MeA), VMH, BNST, and dorsolateral part of the midbrain central gray is increased in subjects who attack conspecific intruders compared to subjects who experience only the odor of conspecifics in their homecage. The AH has reciprocal connections with these areas, and it is suggested that in hamsters the AH is an area of integration in a network of neural sites controlling offensive aggression.

Studies have also provided information as to neural substrates of submissive behavior in the male Syrian hamster. In male hamsters acute defeat activates distinct brain regions and chronic defeat leads to a selective pattern of habituation of immediate early gene expression within a subset of these brain regions. Kollack-Walker, Watson, and Akil (1997) report that in subordinate animals, c-*fos* expression is elevated in regions including the cingulate cortex, lateral septum (LS), amygdalohippocampal area (Ahi),

septohypothalamic nucleus (Shy), dorsal periaqueductal gray (PAG), cuneiform nucleus (CnF), central nucleus of the amygdala (CE), BNST, MPOA, dorsal raphe, locus coeruleus, and various nuclei of the hypothalamus (paraventricular nucleus [PVN], AH, VMH, and the arcuate nucleus). In addition, Kollack-Walker, Don, and Akil (1999) found that chronic defeat is associated with a decrease in c-*fos* expression in the some of these regions (anterior subdivision of the PVN, AH, CE, intermediate subdivision of the LS, SON, Shy) while no change in expression (i. e., no adaptation) is seen in the other regions (AH, CnF, dorsal PVN, and lateral VMN). A decrease in expression may represent habituation of physiological processes. In brain regions where no adaptation of c-*fos* expression was observed, this lack of change could be represent processes that are not as likely to adapt to repeated defeat and may include behaviors associated with defense (Kollack-Walker et al., 1999).

As implied above, the amygdala is appears to be part of a neuroanatomical defense system and this classification would seem in part due to its projections to regions of the hypothalamus and central gray that are involved in the organization of the motor output of this system (Adams, 1979; Davis 2000; LeDoux, Iwata, Cicchetti, & Reis, 1988). At a general level, it has been proposed the amygdala is involved in the analysis and integration of information about the internal and external environment (Aggleton & Mishkin, 1986). It is not surprising, then, that there is a clear line of evidence indicating that the amygdala is involved in responses to unconditioned and conditioned aversive stimuli (Davis, 2000).

Based on its efferent and afferent projections, the amygdala appears to be ideally positioned to mediate the formation and expression of conditioned aversive responses (LeDoux, 2000; Maren & Fanselow, 1996). The basolateral amygdala (BLA), which receives sensory information from cortical and subcortical structures (Charney, Grillon, Bremner, 1998; LeDoux, Cicchetta, Xagorais, & Romanski, 1990), is purported to be a

key area for conditioned and unconditioned stimulus (CS-US) associations during aversive, or fear, conditioning (Fanselow & LeDoux, 1999). The central nucleus of the amygdala (CE), which receives input from the BLA, is regarded as the output system of the amygdala and projects to many nuclei that are involved in behavioral and physiological responses of fear and anxiety (Davis, 2000, Maren & Fanselow, 1996; LeDoux, Iwata, Cicchetti, & Reis, 1988).

The CE has been implicated in autonomic (Henke, 1985; Kapp, Gallagher, Frysinger, & Applegate, 1981), neuroendocrine (Roozendaal, Koolhaas, & Bohus, 1991a,b; Van de Kar, Piechowski, Rittenhouse, & Gray, 1991), and behavioral stress responses (Goldstein, Rasmusson, Bunney, & Roth, 1996; Lee & Davis, 1997; Roozendaal et al., 1991b; Swiergiel, Takahashi, & Kalin, 1993). Specifically, the CE appears to modulate a variety of unconditioned and conditioned responses to aversive stimuli. Lesions of the CE attenuate vagal-mediated (Kapp, Frysinger, Gallagher, & Haselton, 1979) and neuroendocrine responses to acute stressors (Beaulieu, Di Paolo, & Barden, 1986; Marcilhac & Siaud, 1996; Prewitt & Herman, 1994; Van de Kar et al., 1991), vagal-mediated and neuroendocrine conditioned responses (Roozendaal, Koolhaas, & Bohus, 1992; Van de Kar et al., 1991), and unconditioned and conditioned behavioral responses to aversive stimuli (Amoranth, LeDoux, & Nader, 2000; Goldstein et al., 1996; Kemble, Blanchard, & Blanchard, 1990; Roozendaal et al., 1991b, Kim & Davis, 1993).

Unconditioned and conditioned behavioral, autonomic, and hormonal responses used as tests or signs of fear and anxiety (Davis, 1992, 1997, 2000) are comparable to responses seen during, and in response to, aversive social contexts such as social defeat. If the CE is involved in certain unconditioned and conditioned fear responses to aversive situations/stimuli, then the CE may be involved in the mediation of responses to social defeat. As previously indicated, Kollack-Walker, Watson, and Akil (1997) found a

selective increase of c-*fos* messenger ribonucleic acid (mRNA) labeling in the CE in hamsters who experienced social defeat as compared to dominant or control hamsters.

In general, lesions of the amygdala have increased, decreased, or had no effect on agonistic behavior. This confusion may be due to differences in context, experimental paradigm, choice of dependent measure, and particular amygdaloid nuclei that were targeted (Blanchard & Takahashi, 1988; Bush & Barfield, 1974; Seigal & Edinger, 1983). Similarly, in subjects with CE lesions, unconditioned behavioral responses to aversive stimuli have been attenuated (Blanchard & Blanchard, 1972; Kim, Rison, & Fanselow, 1993; Roozendaal et al., 1991a) or have been left unchanged (Goldstein et al., 1996; Holahan & White, 2002). With respect to amygdaloid involvement in agonistic behavior in Syrian hamsters, Bunnell et al. (1970) found that large amygdaloid lesions decreased the amount of social contact with conspecifics. Following these lesions, subjects with defeat experience were less submissive, and subjects with "winning" experience were less dominant. In addition, lesion subjects that were socially inexperienced showed less aggressive or submissive behavior compared to preoperatively aggressive or submissive lesioned animals, respectively.

The purpose of this study was to investigate the involvement of the CE in neuroendocrine and behavioral responses associated with social defeat and conditioned defeat. Two experiments were conducted in which male golden hamsters received electrolytic lesions targeting the CE, or they served as sham lesion or home-cage controls. Subjects were included in either the acute defeat (Experiment 1) or the conditioned defeat (Experiment 2) experiment.

In Experiment 1, subjects were subjected to one 10 min resident-intruder encounter or served as baseline controls. To facilitate social defeat, subjects were introduced into an aggressive animal's home cage. Single-trial social defeat permitted the assessment of the amygdaloid lesions on behavioral and neuroendocrine (ACTH and

cortisol) responses to acute defeat. It was hypothesized that acute defeat would increase plasma levels of ACTH and cortisol above baseline levels. In addition, it was hypothesized that CE lesions would attenuate the duration of submissive-defensive behavior and the defeat-induced increase in ACTH and cortisol.

Experiment 2 assessed the role of the CE in conditioned defeat. Subjects were placed in one of two conditions: conditioned defeat (CD) or no defeat (ND). Each condition included lesioned and non-lesioned subjects. On Day 1, subjects in the conditioned defeat condition (CD) were exposed to an aggressive resident (10 min), while subjects serving as novel cage controls (ND) were placed in an aggressor's empty home cage (10 min). Twenty-four hours later, subjects in both conditions were exposed to a non-aggressive intruder (10 min). It was hypothesized that CE lesions in subjects with prior defeat experience would decrease the duration of submissive-defensive behavior and/or increase the duration of aggressive behavior in response to a non-aggressive intruder. It was also hypothesized that subjects with defeat experience would show an augmented neuroendocrine (ACTH and cortisol) response to the non-aggressive intruder which would be attenuated in subjects with CE lesions.

METHOD

Animals and Housing Conditions

For Experiments 1 and 2, adult male Syrian hamsters (*Mesocricetus auratus*), weighing 120-130 g at the beginning of the study, were obtained from Charles River Laboratories. Two weeks prior to testing and for the duration of each study, these animals were individually-housed in a temperature controlled colony room (20 ± 2°C) on a 14:10 hr light-dark (L:D) cycle with the lights off at 1100 hr. Additional hamsters weighing 150 –180 g at the beginning of the study were used as resident aggressors for acute defeat sessions, and hamsters weighing 100-110 g at the beginning of the study were used as non-aggressive stimulus animals during conditioned defeat testing. Resident aggressors were individually housed. Non-aggressive intruders were group housed (4-5 animals/cage). All animals were housed in Plexiglas cages (20 x 40 x 20 cm) with wire mesh tops. Pelleted rodent chow (Purina Mills, St. Louis, MO) and water were available ad libitum. All procedures and protocols were approved by the University of Georgia and Georgia State University Institutional Animal Care and Use Committees.

Surgical Procedures

Following anesthetization using sodium pentobarbital (Nembutal, 90 mg/kg, ip), surgical subjects were placed in the stereotaxic instrument. After an incision was made to expose the skull, the head was leveled with respect to lambda and bregma (Rook, 1973). Lesions were made with epoxylate-coated tungsten electrodes connected to a lesion maker (Grass Instruments, West Warwick RI). Electrolytic lesion parameters (coordinates, current, and time) are listed on Table 1. Sham lesions were made by using the same coordinates, but the current was not turned on. Post-operatively, subjects were monitored daily during which time the general condition of each animal

Table 1

Lesion Coordinates

Experiment	Anterior/Posterior	Medial/Lateral	Dorsal/Ventral	Current (mA)	Duration (sec)
1	-0.2	± 3.7	-6.3	2.10	20
	-0.3	± 3.6	-6.2	1.75	20
	0, -0.3	± 3.7	-6.2	1.25	15
2	0, -0.3	± 3.7	-6.2	1.25	15
	+0.3, -0.1	+3.6, -3.7	6.1, 6.0	1.25	15
	+0.3, -0.1	± 3.6	6.1, 6.0	1.25	15

was evaluated. Subjects were allowed a minimum of 14 days post-operative recovery and were handled daily for 7-10 days before testing began.

Acute and Conditioned Defeat

Stimulus animals. To increase the probability of aggression and to decrease variability in subject behavior, animals that were heavier and individually housed were use as aggressive residents to defeat subjects. Additionally, these animals were pre-screened for aggression through multiple interactions with non-aggressive intruders, and animals that were determined to attack reliably were used as aggressive residents. Animals selected as aggressors were individually housed throughout the study, a technique that has been used to increase aggression levels in golden hamsters (Brain, 1972a; Landau, 1975; Lerwill & Makings, 1971). Aggressive residents were used in acute defeat trials in Experiments 1 and 2.

Animals used as non-aggressive intruders were group-housed and, on average, smaller than the experimental subjects. When placed in the home cage of a previously-defeated resident, non-aggressive intruders generally pay little attention to other animals that are present, but are able to evoke submissive/defensive behaviors from the previously-defeated residents (Potegal et al., 1993). Non-aggressive intruders were used in conditioned defeat testing in Experiment 2.

Social Defeat/Testing. Social defeat trials began a minimum of 14 days post-operatively. Trials took place during the first 3 hours of the dark phase of the LD cycle to minimize the circadian variation of the dependent measures (Albers, Yogev, Todd, & Goldman, 1987). All trials were conducted under dim illumination. During the course of a social encounter if an animal received a bleeding injury, the trial was ended and the animal was removed from the study.

Behavioral tests were recorded on VHS tape, transferred to CD-ROM, and scored blindly using Noldus Observer (version 4; Noldus Information Technology, Wageningen,

Netherlands). Classes of behavior from a modified version of the Free Interaction Social Test inventory (FIST) were recorded as total duration in seconds. See Table 2 for FIST.

Blood Collection and Plasma Preparation

Following acute trials in Experiment 1 and final trials in Experiment 2, trunk blood was collected after rapid decapitation. Blood collection was counterbalanced across all groups. For subjects in social interaction trials, blood was collected within approximately 5 min of the end of the final trial and was dispensed into heparinized beakers (.05 ml). The blood was spun in a refrigerated centrifuge at 4000 rpm for a minimum of 10 min. Plasma was separated and stored at -20 C^{o} until radioimmunoassays were performed.

Radioimmunoassays

Radioimmunoassays for ACTH and cortisol were conducted as in Huhman et al., (1990). ACTH was assayed with a radioimmunoassay kit purchased from DiaSorin Inc (Stillwater, Minnesota). To date, the cross-reactivity of the rabbit-anti ACTH antibody with hamster ACTH has not been reported. But, this ACTH antibody has been known to cross-react with many other vertebrates' ACTH (INCSTAR Corp., unpublished data). Assay sensitivity was 15 pg/ml. The intraassay coefficient variation was 9.4%. All samples were run together in the same assay. Plasma was assayed for cortisol using a Coat-A –Count kit purchased from Diagnostics Products Corporation (Los Angeles, California). Assay sensitivity was 0.15 μg/dl. The intraassay coefficient was 4.6%. All samples were run together in the same assay.

Histology

Following rapid decapitation, brains from all subjects were excised and preserved in a 10% formalin solution for a minimum of 48 hr. Using a vibrating microtome, the brains were sectioned at 40 μm. Sections were stained using either cresyl violet or

Table 2

The Free Interaction Social Test (modified)

Nonsocial
Locomotor/exploratory
Self -grooming
Picking up, pouching, piling, arranging or carrying nest materials
Picking up, pouching, piling, arranging, carrying, or eating food
Sleeping
Social
Attend (turn head, or head and body toward opponent)
Sniff opponent's head or body (requires physical contact)
Approach (move toward opponent)
Aggressive
Upright and underneath attack
Bite
Chase
Submissive/Defensive
Teeth Chatter
Flag (turn head, or head and body away from opponent)
Tail lift with or without adduction of hindleg
Freeze (lying on back, immobile)
Attempted escape from cage (climbing, clinging to wall)
Flee (rapid movement away from opponent)
Upright and side defensive posture

Note. From Sodetz and Bunnell (1970), modified.

thionin stain. An independent rater judged lesion placement in random slides and determined post-hoc grouping of subjects according to lesion placement and size.

Experiment 1: CE lesion and Acute Defeat

Experiment 1 tested the hypothesis that the CE is involved in the expression of submissive/defensive behavior and the ACTH and cortisol response to a 10 min social-defeat encounter. Subjects were randomly assigned to one of three surgical conditions: (a) CE lesion, (b) operated (sham lesion) control, or (c) non-operated control. Half of the subjects in each condition were randomly assigned to one of two treatments conditions, acute defeat or baseline control. Baseline control subjects were removed from the cage only for handling and weighing.

Acute social conflict trials began 14 days post-operatively. Subjects in the acute defeat condition were placed in the cage of an aggressive male resident for 10 min. Each subject was tested only once and social defeat trials were recorded on VHS. Except for handling, baseline control animals remained in their cages for the duration of the experiment.

Experiment 2: CE Lesion and Conditioned Defeat

Experiment 2 tested the hypothesis that the CE modulates conditioned defeat behavior and ACTH and cortisol responses of previously-defeated animals during a subsequent 10 min social encounter with a non-aggressive intruder. A statistically significant decrease in the duration of submissive/defensive behaviors and/or the display of aggression in CD subjects denotes a reduction in, or blockade of, conditioned defeat, respectively (Potegal et al., 1993). Subjects were randomly assigned to one of three surgery conditions: (a) CE lesion, (b) operated (sham lesion) control, (c) non-operated control. Half of the subjects in each surgery condition were randomly assigned to one of two treatment conditions, conditioned defeat (CD) or no defeat (ND).

On Day 1 of Experiment 2, subjects in the CD condition experienced acute defeat using procedures identical to the acute defeat condition in Experiment 1. ND animals were placed in an aggressor's empty home cage for 10 min. Twenty-four hours later, a non-aggressive stimulus animal was placed in the home cages of both CD and ND subjects for 10 min. Social defeat and conditioned defeat trials were recorded on VHS.

Behavioral and Statistical Analysis

In Experiments 1 and 2 the total duration (sec) of each class of behavior displayed (submissive/defensive, social, aggressive, nonsocial) was determined using Noldus Observer. ACTH and cortisol measures were determined via RIAs. For all dependent measures in Experiments 1 and 2, differences between the non-operated and operated control animals were assessed using *t*-tests. When a test indicated that there was no significant difference between these two control conditions, the data were pooled into one control condition. All data were subjected to Levene's test of homogeneity.

For Experiment 1, the duration data for each behavior class for acute defeat subjects was analyzed using one-way between-subjects analysis of variance (ANOVA) with lesion as the between-subjects factor. ACTH and cortisol responses were analyzed using 4 X 2 between-subjects ANOVAs with lesion (small, large, unilateral, control) and condition (acute defeat, baseline control) as between subjects factors. Statistically significant differences were analyzed further using Bonferoni-Dunn post-hoc tests. Unless noted otherwise, statistical significance was ascribed at $p < .05$.

For Day 1 of Experiment 2, the duration data for each behavior class for CD subjects and aggressive behavior in resident aggressors were analyzed using a one-way between-subjects analysis of variance (ANOVA) with lesion as the between-subjects factor. Statistically significant differences were analyzed further using Bonferoni-Dunn post-hoc tests. For Day 2 of Experiment 2, the duration data for each behavior class, ACTH responses, and cortisol responses for CD and ND subjects were analyzed using 2

X 2 between-subjects ANOVAs with lesion (amygdala lesion, control) and condition (CD, ND) as the between-subjects factors. Statistically significant differences were analyzed further using multiple t's/Fisher LSD post-hoc tests. Unless noted otherwise, an alpha level of .05 was used for all statistical tests.

RESULTS

In Experiment 1, hamsters were placed in the home cage of an aggressive resident or served as baseline controls for neuroendocrine measures. Post-hoc grouping of subjects yielded small, large, unilateral and control amygdala lesion groups. No significant differences among groups were observed in submissive/defensive, social, and nonsocial behavior. Hamsters with unilateral lesions showed significantly more aggressive behavior compared to all other lesion groups. For ACTH and cortisol, the main effect of condition, but not lesion, was significant. On Day 1 of Experiment 2 subjects either experienced defeat by an aggressive resident or were placed in an empty aggressors home cage for the same amount of time. Twenty-four hours later, a non-aggressive hamster was placed in the home cages of all subjects. Post-hoc grouping of subjects within these conditions yielded amygdala lesion and control lesion groups. Amygdala lesions did not alter behavioral responses to an aggressor during initial defeat and did not produce differences in the intensity of aggression received during initial defeat. Amygdala lesions did attenuate submissive behavior in previously-defeated hamsters in response to non-aggressive intruders. Amygdala lesions did not alter levels of aggressive behavior, but aggressive behavior in previously-defeated hamsters was significantly lower than hamsters with no defeat experience. In non-defeated hamsters, amygdala lesions decreased social behavior and increased nonsocial behavior. The increase in nonsocial behavior appeared to be due to an increase in locomotor/exploratory behavior. Plasma cortisol and ACTH levels were similar across all groups.

Experiment 1: Effects of Lesions of the CE/BLA on Behavioral and Hormonal Responses to Acute Defeat

Histology. In Experiment 1, lesion placement for two animals could not be verified and a total of 6 animals died due to anesthesia. ACTH data from two subjects

were not used due to excessively high ACTH responses (> 3 *SD* + *M*). In the acute defeat condition, nine subjects had large lesions (≥½ area of a nucleus at the site of largest damage) of the CE or CE/BLA area. Six subjects had small lesions (< ½ of the total area of the CE) and of these six subjects, four subjects had small lesions (< ½) of the basolateral amygdala (BLA; 3 bilateral; 1 unilateral). Additionally, four subjects had either unilateral lesions of the CE or CE/BLA area. So, a total of 30 subjects were included in the acute defeat condition for data analysis for cortisol and all behavioral dependent measures: Large (*n* = 9); Small (*n* = 6), Unilateral (*n* = 4), and Control (*n* = 11). Twenty-eight subjects were included in the ACTH data analysis: Large (*n* = 9); Small (*n* = 5), Unilateral (*n* = 3), and Control (*n* = 11).

In the baseline control condition, six subjects had small lesions of the CE/BLA. Four subjects had unilateral lesions of the CE or CE/BLA area. Additionally, nine subjects had bilateral lesions of the CE/BLA area. Six of these subjects had large bilateral CE/BLA lesions (≥ ½ of both nuclei at the site of the largest lesion). A total of 31 subjects were in the BC condition for data analysis: Large (*n* = 9); Small (*n* = 6), Unilateral (*n* = 5), and Control (*n* = 12). In Experiment 1, some damage to the internal capsule, globus pallidus, intraamygdaloid bed nucleus of the stria terminalis, interstitial nucleus of the posterior limb of the anterior commissure, anterior, the lateral, cortical, medial, basomedial, and posteriolateral nuclei of the amygdala, amygdalostriatal transition zone and the ventral and dorsal endipiriform was observed in various subjects but this damage was distributed across groups. In addition, damage to the CE was restricted to mainly the lateral region, leaving most of the medial region intact.

Figure 1 provides serial coronal plates through the extent of the central nucleus of the amygdala and appropriate nuclei are labeled. For the acute defeat condition, histological reconstructions of the smallest and largest lesions in each group (large, small, or unilateral lesion groups) are shown in Figure 2. For the baseline condition,.

Figure Caption

Figure 1. Serial coronal plates through the extent of the central nucleus of the amygdala with appropriate nuclei labeled (Morin & Wood, 2001).

-0.3mm

-0.6mm

-0.9mm

-1.2mm

-1.5mm

-1.8mm

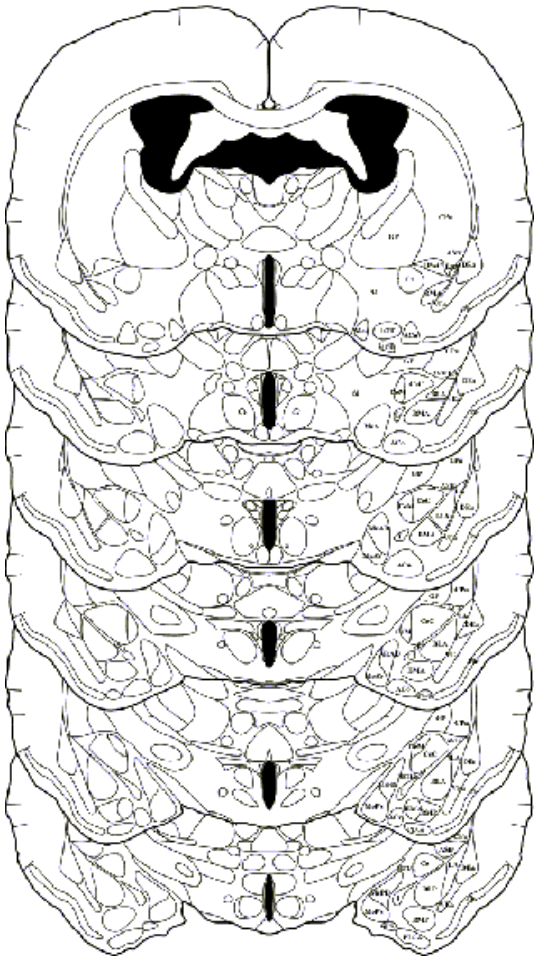
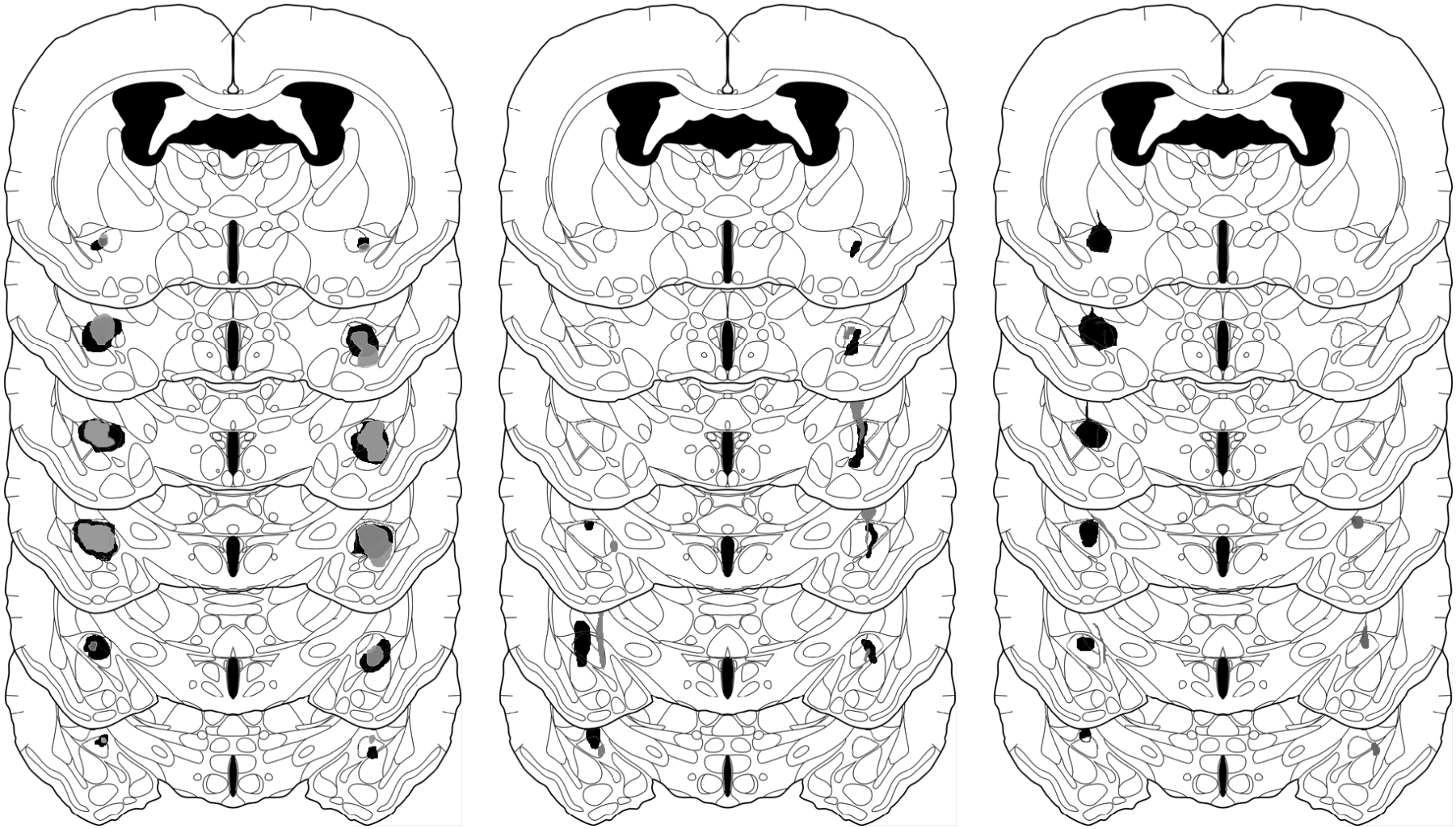


Figure Caption

Figure 2. Histological reconstructions of the smallest (gray/stippled) and largest (black) lesions in each group (large [≥½ area of a nucleus at the site of largest damage of the CE or CE/BLA area], small [< ½ of the total area of the CE or CE/BLA], or unilateral [CE or CE/BLA area] lesion groups) for the acute defeat condition in Experiment 1.



histological reconstructions of the smallest and largest lesions in each group (large, small, or unilateral lesion groups) are shown in Figure 3

Agonistic and Neuroendocrine Responses. The control group was comprised of non-operated and sham controls. There were no differences between these two groups on any of the dependent measures ($p > .05$). Therefore, all data in sham and non-operated control conditions were pooled to form one control condition.

For all dependent measures homogeneity of variance was tested using Levene's test of homogeneity. For nonsocial, social, and aggressive behavior and for ACTH, homogeneity of variance was violated ($p < .05$). Therefore, alpha was set at .01 to correct for the increase in the probability of a Type 1 error associated with heterogeneity of variance (Sheskin, 2000).

No significant differences were observed in submissive/defensive ($F_{(3, 26)} = 2.721$, $p > .05$), social ($F_{(3, 26)} = 1.165$, $p > .01$) and nonsocial ($F_{(3, 26)} = 3.742$, $p = .023$) behavior (Figures 4-6). A one-way ANOVA indicated a significant difference in aggressive ($F_{(3, 26)} = 6.079$, $p < .01$) behavior (Figure 7). Bonferroni-Dunn post-hoc tests revealed that aggressive behavior in unilateral lesioned animals was significantly higher than in animals with large, small, and no lesions.

For ACTH, the 4 X 2 ANOVA revealed a significant effect of condition ($F_{(1, 51)} = 164.933$, $p < .01$). Neither a significant main effect of lesion ($F_{(3, 52)} = 3.194$, $p = .031$) nor a significant lesion by control interaction ($F_{(3, 51)} = .3851$, $p > .01$) was found. Animals that had experienced acute defeat had higher ACTH responses compared to animals that were used as baseline controls (Figure 8). For cortisol, the 4 X 2 ANOVA revealed a significant main effect of condition ($F_{(1, 54)} = 77.164$, $p < .05$). The main effect of lesion ($F_{(3, 54)} = .800$, $p > .05$) and the lesion by condition interaction ($F_{(3, 54)} = .216$, $p > .05$) were not significant. Animals that experienced acute defeat had higher cortisol responses compared to animals that were used as baseline controls (Figure 9).

Figure Caption

Figure 3. Histological reconstructions of the smallest (gray/stippled) and largest (black) lesions in each group (large [≥½ area of a nucleus at the site of largest damage of the CE or CE/BLA area], small [< ½ of the total area of the CE or CE/BLA], or unilateral [CE or CE/BLA area] lesion groups) for the baseline condition in Experiment 1.

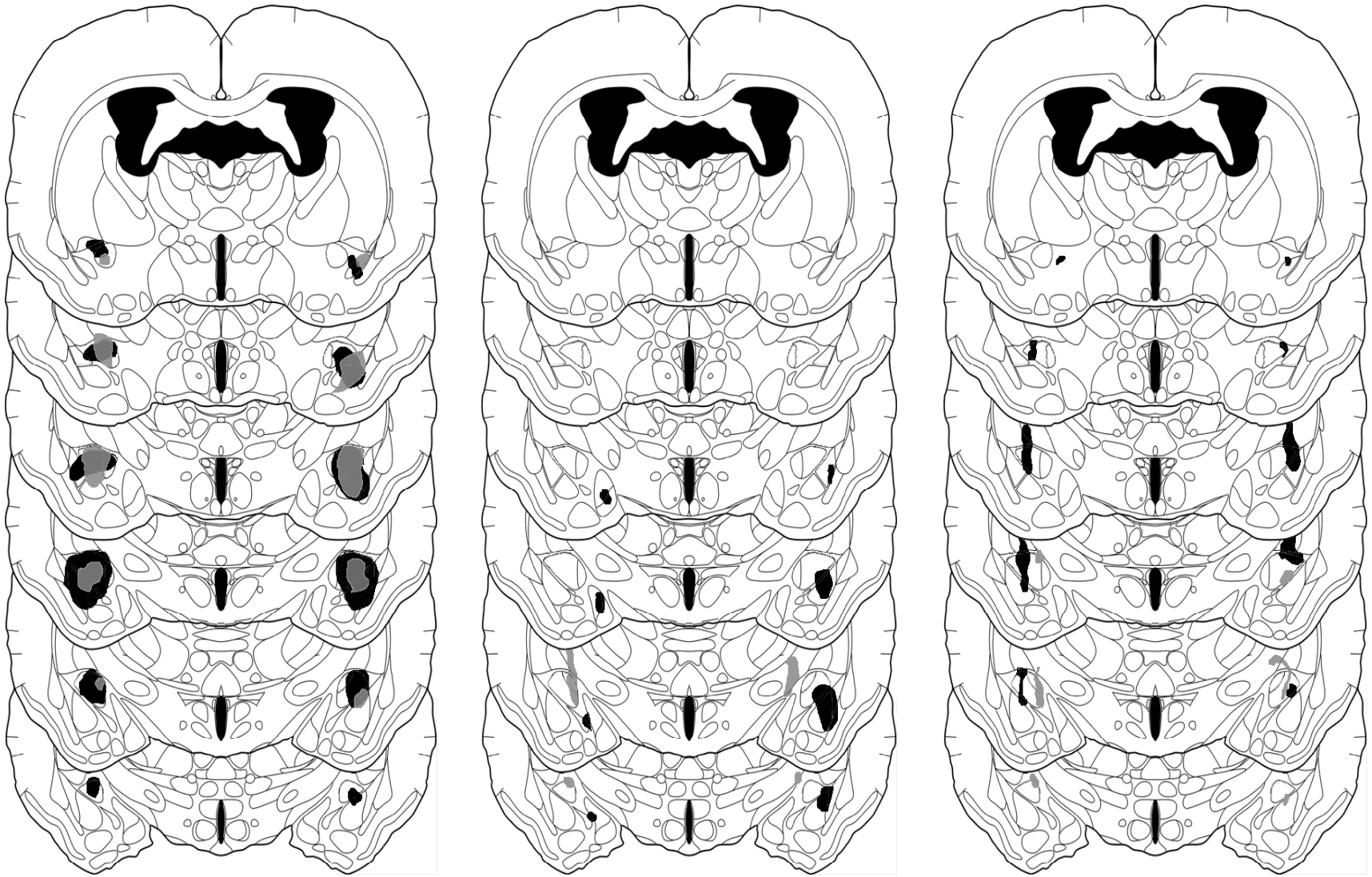


Figure Caption

Figure 4. Total duration (*M* + *SEM*) of submissive behavior displayed by large (*n* = 9), small (*n* = 6), unilateral (*n* = 4) and control (*n* = 11) amygdala-lesioned animals toward resident aggressors during a 10-min encounter. No significant differences were found.

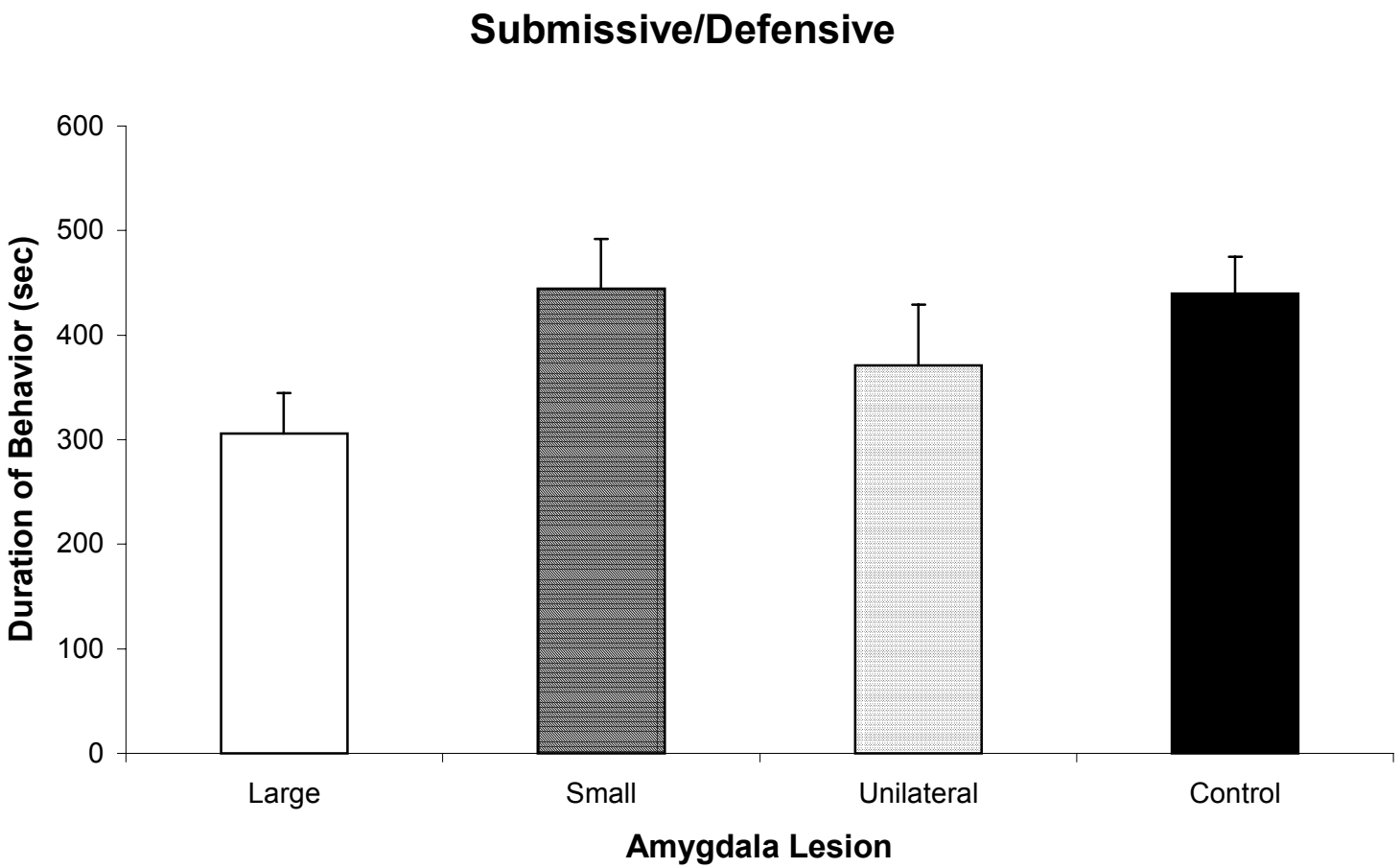

Submissive/Defensive
Duration of Behavior (sec)
600
500
400
300
200
100
0
Large
Small
Unilateral
Control
Amygdala Lesion

Figure Caption

Figure 5. Total duration (*M* + *SEM*) of social behavior displayed by large (*n* = 9), small (*n* = 6), unilateral (*n* = 4) and control (*n* = 11) amygdala-lesioned animals toward resident aggressors during a 10-min encounter. No significant differences were found.

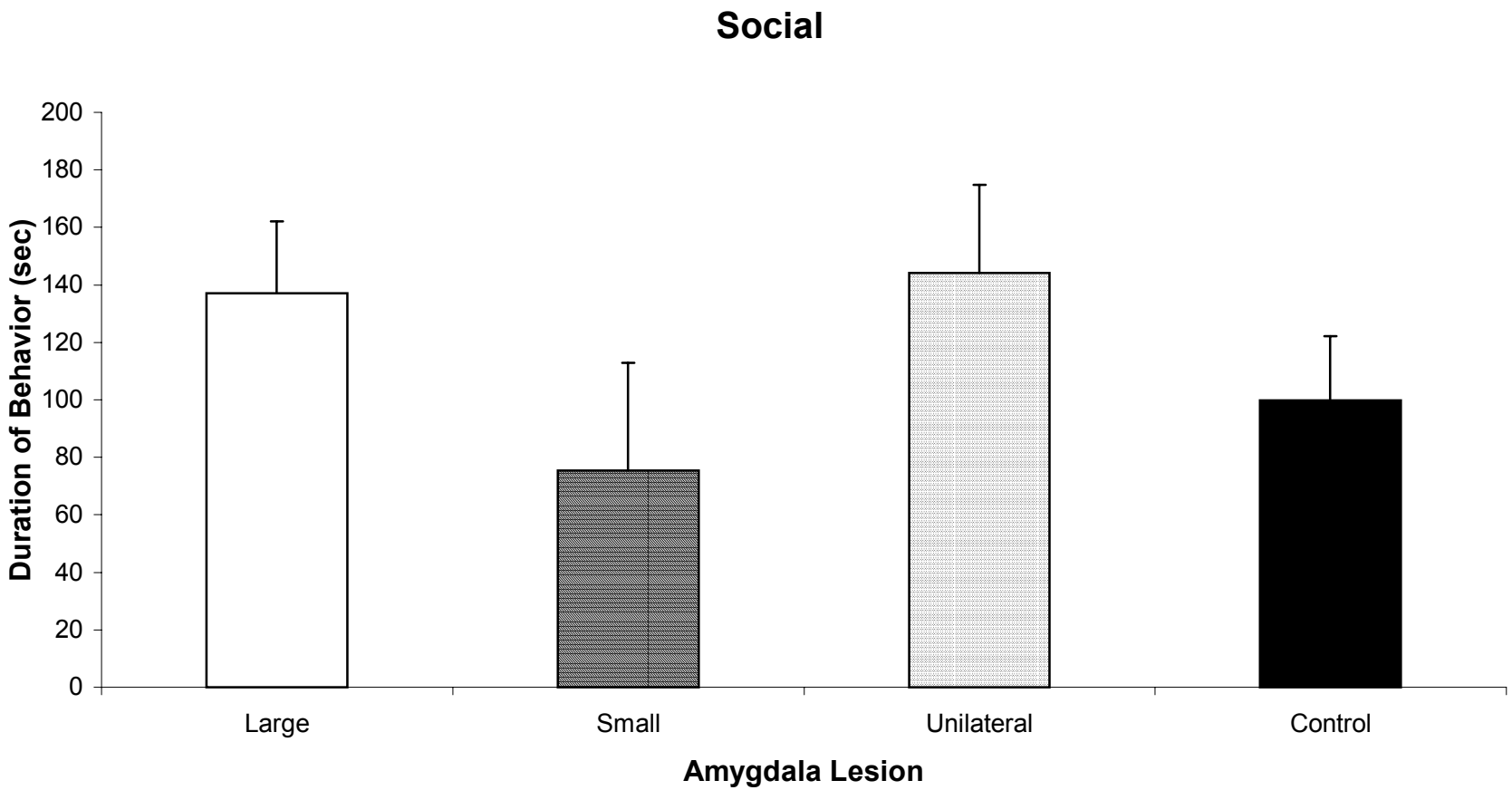

Social
Duration of Behavior (sec)
200
180
160
140
120
100
80
60
40
20
0
Large
Small
Unilateral
Control
Amygdala Lesion

Figure Caption

Figure 6. Total duration (*M* + *SEM*) of nonsocial behavior displayed by large (*n* = 9), small (*n* = 6), unilateral (*n* = 4) and control (*n* = 11) amygdala-lesioned animals toward resident aggressors during a 10-min encounter. No significant differences were found.

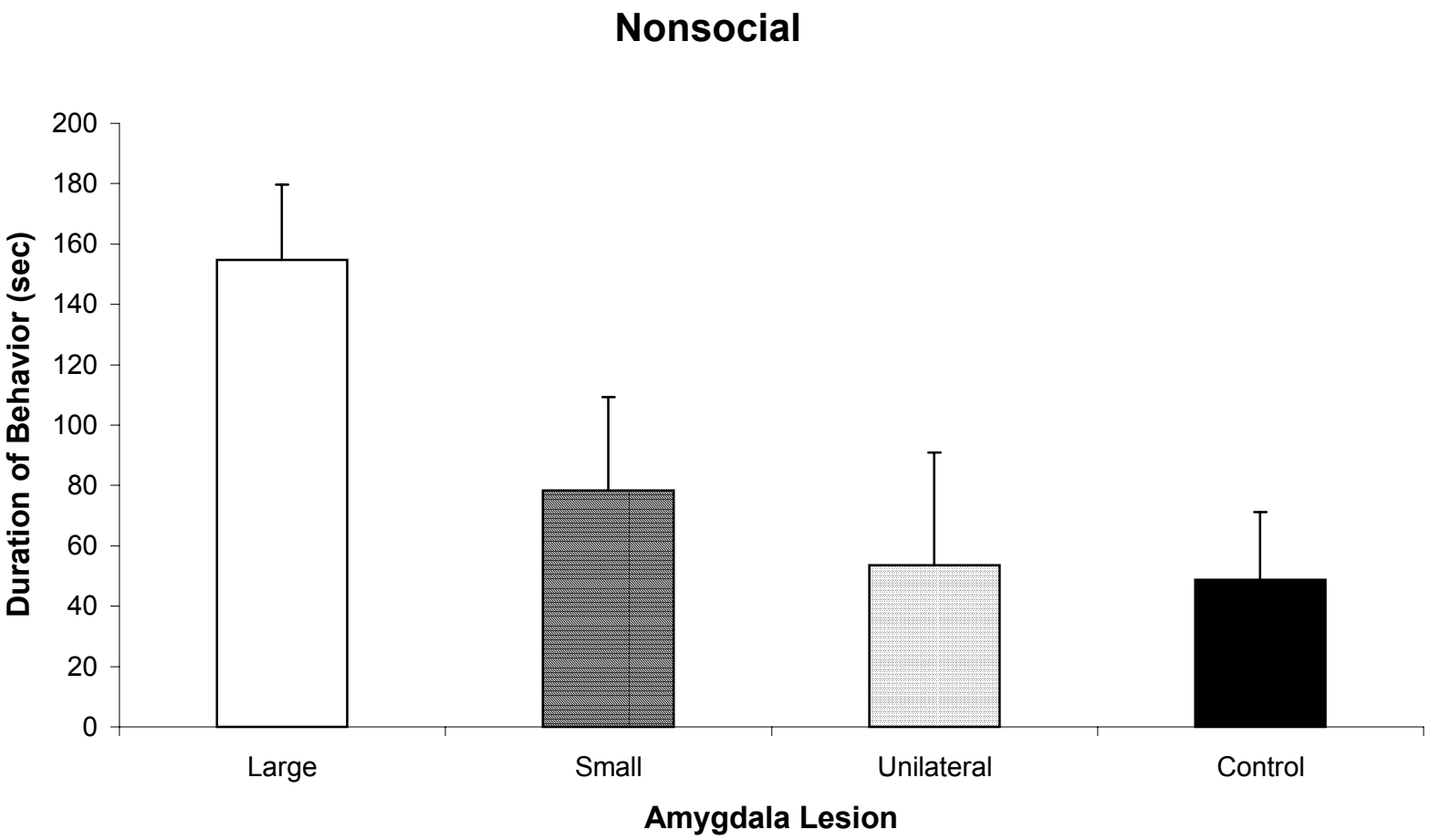

Nonsocial
Duration of Behavior (sec)
200
180
160
140
120
100
80
60
40
20
0
Large
Small
Unilateral
Control
Amygdala Lesion

Figure Caption

Figure 7. Total duration (*M* + *SEM*) of aggressive behavior displayed by large (*n* = 9), small (*n* = 6), unilateral (*n* = 4) and control (*n* = 11) amygdala-lesioned animals toward resident aggressors during a 10-min encounter. *Significantly greater than all other groups, $p < .05$.

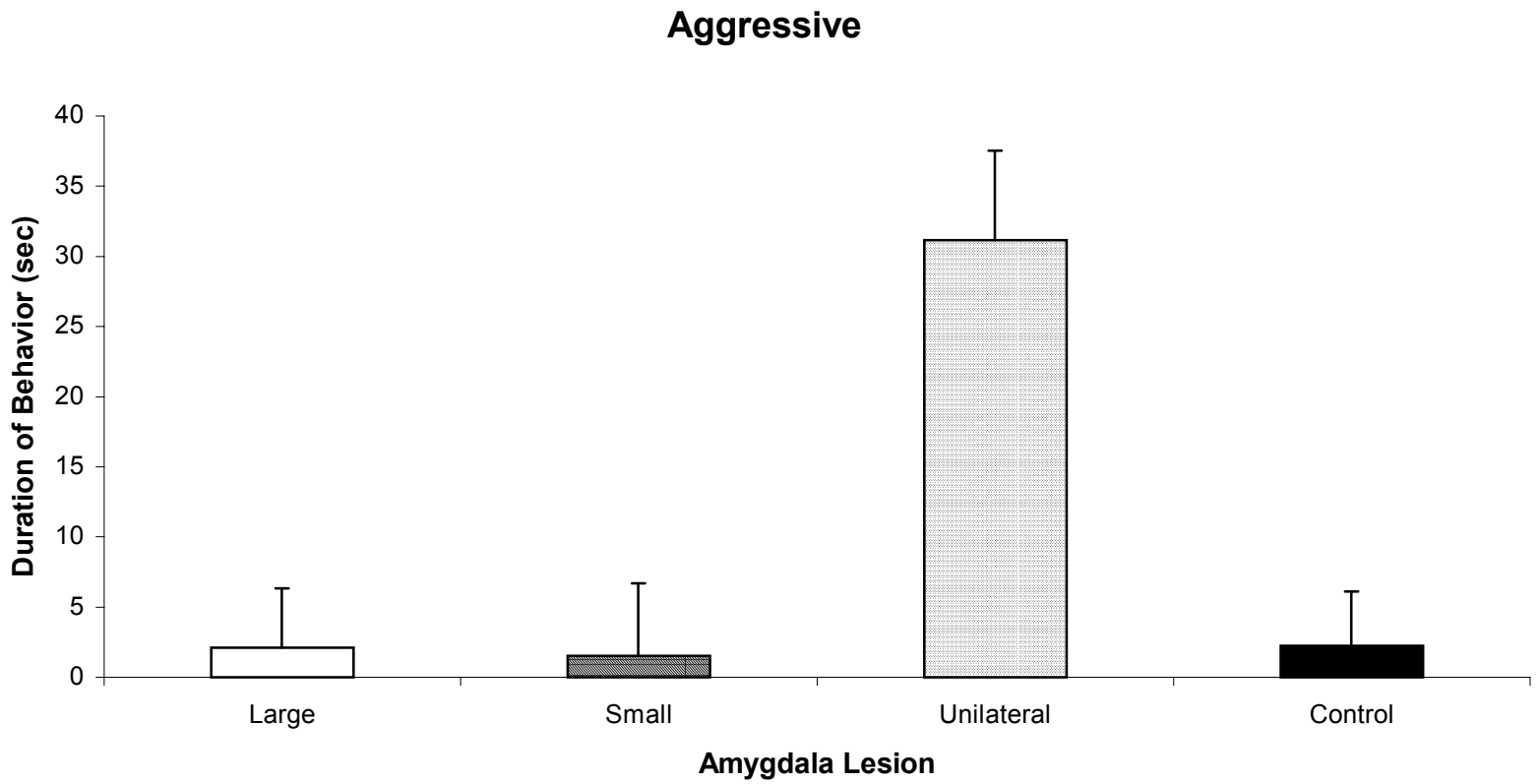

Aggressive
Duration of Behavior (sec)
40
35
30
25
20
15
10
5
0
Large
Small
Unilateral
Control
Amygdala Lesion

Figure Caption

Figure 8. Plasma ACTH (*M* + *SEM*) in animals that experienced acute defeat (amygdala [*n* = 17] and control [*n* = 11]) lesions) or served as baseline controls (amygdala [*n* = 20] and control [*n* = 12]) lesions). *Significantly greater than baseline, $p <$. 01.

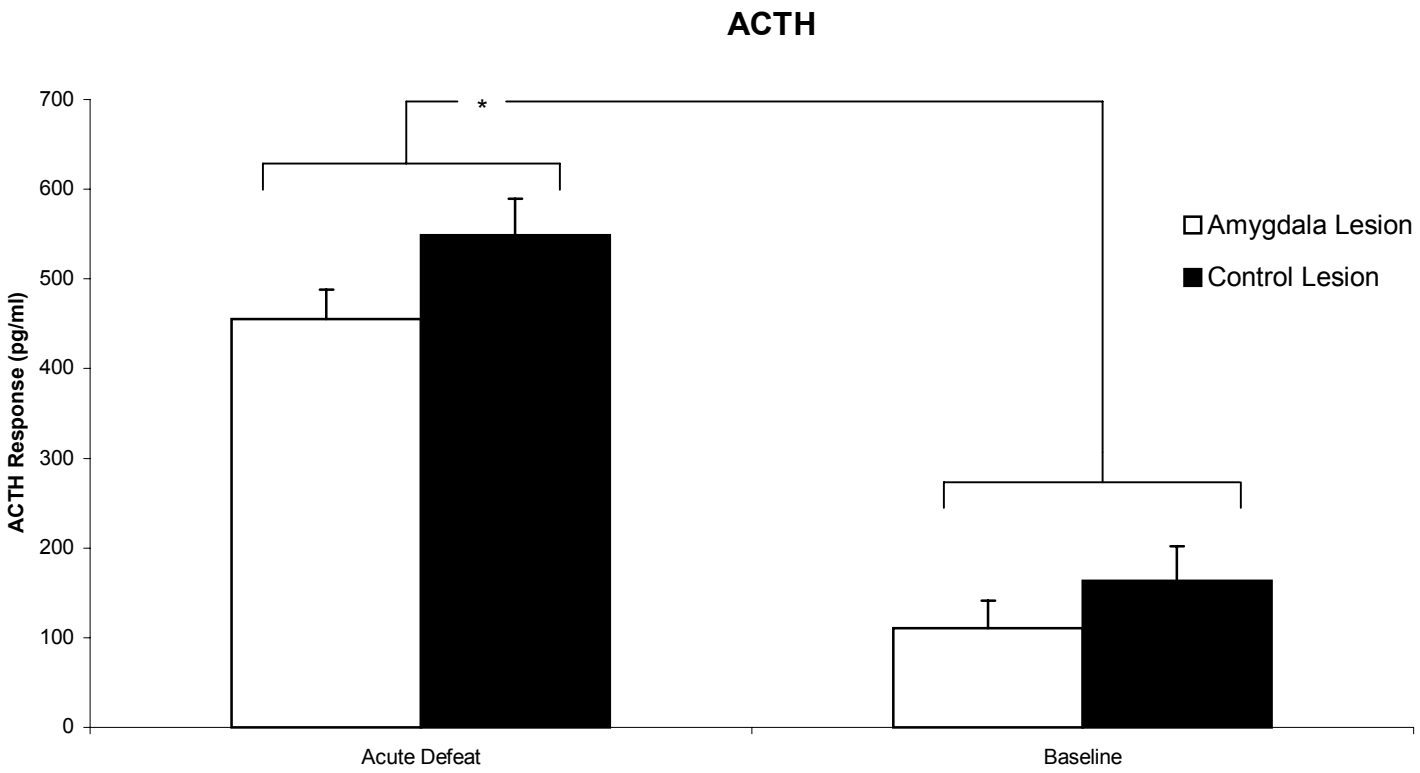
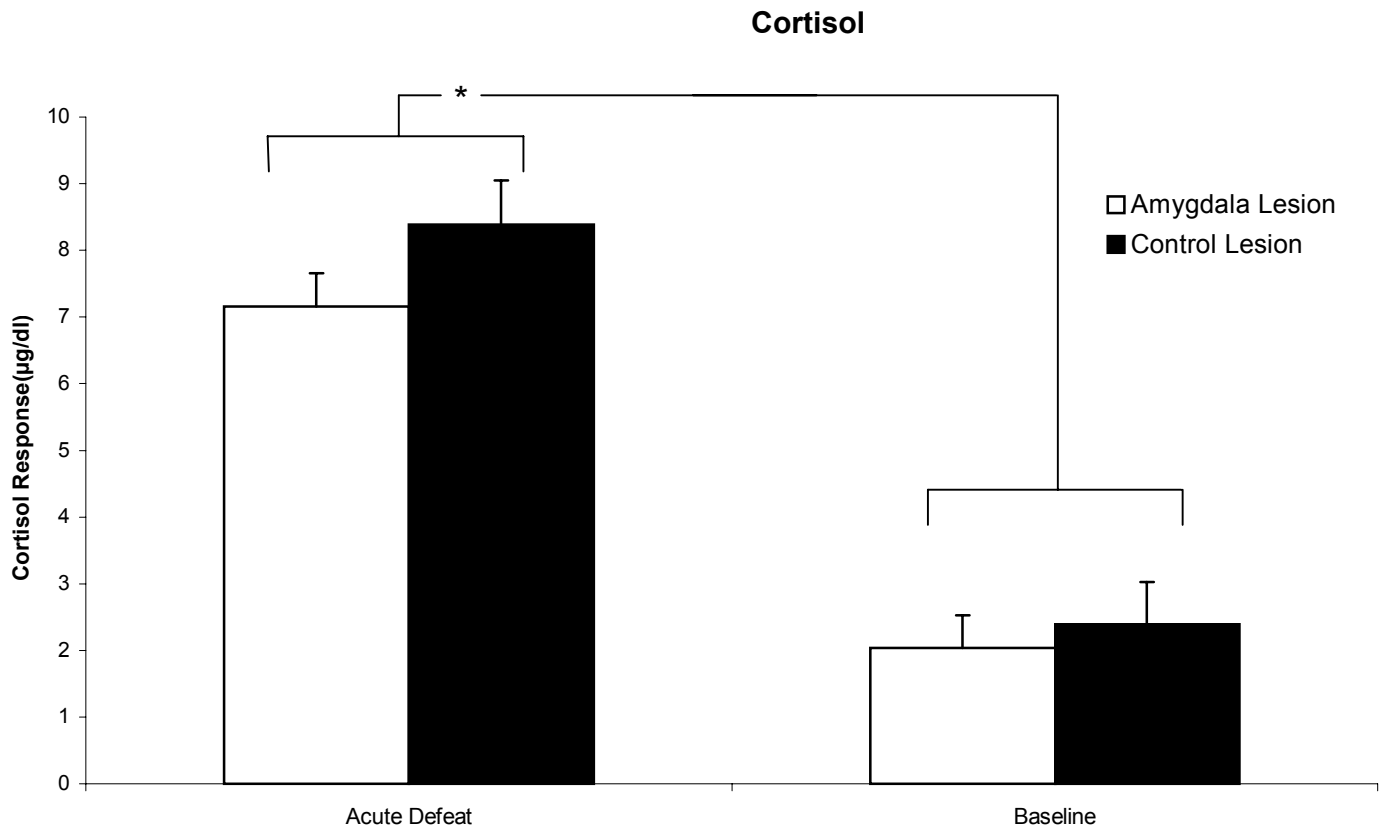

ACTH
ACTH Response (pg/ml)
700
600
500
400
300
200
100
0
*
Amygdala Lesion
Control Lesion
Acute Defeat
Baseline

Figure Caption

Figure 9. Plasma cortisol (*M* + *SEM*) in animals that experienced acute defeat (amygdala [*n* = 19] and control [*n* = 11] lesions) or served as baseline controls (amygdala [*n* =20] and control [*n* = 12] lesions). *Significantly greater than baseline, $p < .05$.


Cortisol
*
10
9
8
7
6
5
4
3
2
1
0
Cortisol Response(µg/dl)
Amygdala Lesion
Control Lesion
Acute Defeat
Baseline

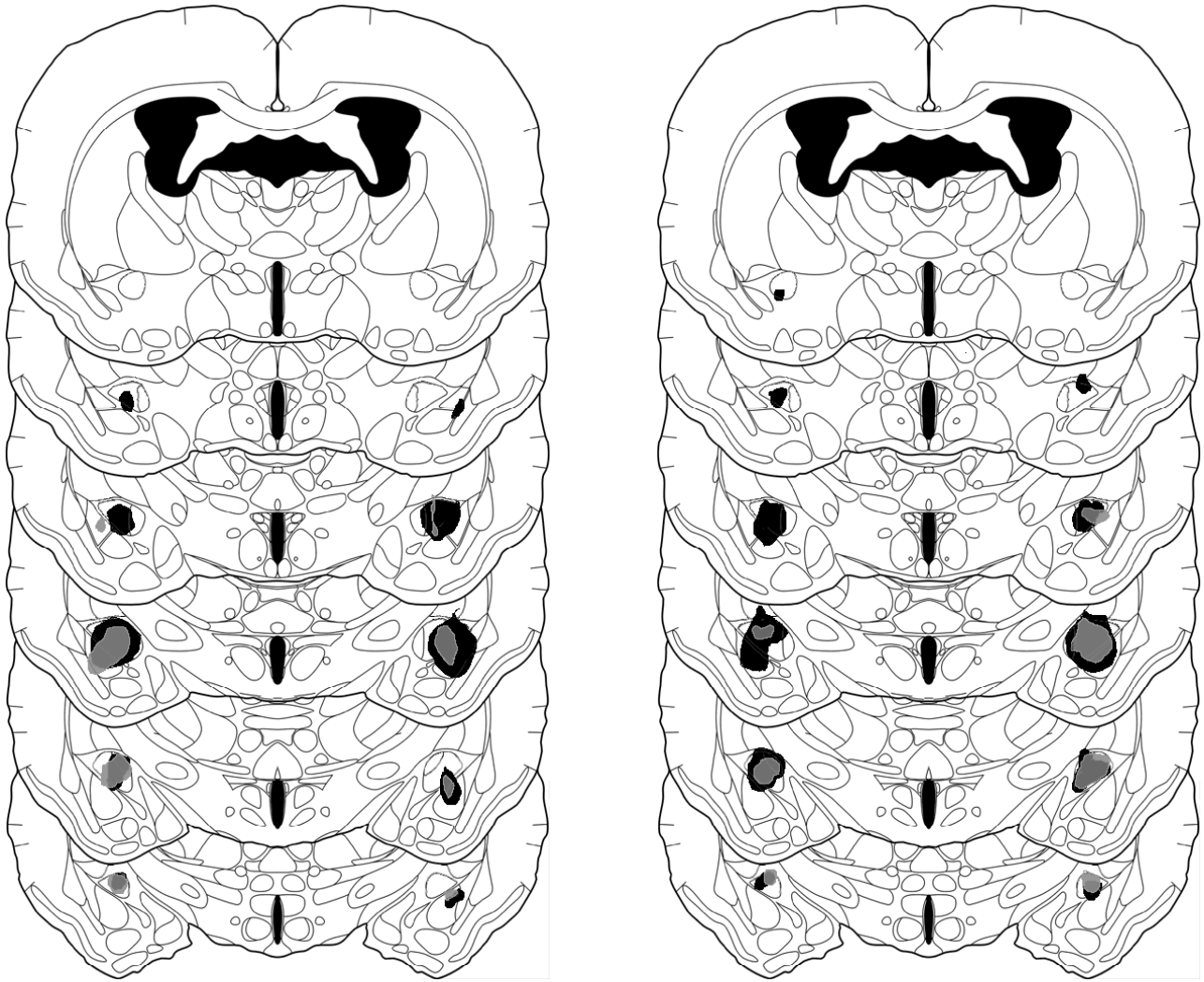
Experiment 2: Effects of Lesions of the CE/BLA on Behavioral and Hormonal Responses to Conditioned Defeat.

Histology. Four animals were removed from the experiment during the acute defeat trials due to a bite wound; no data from these animals were used. Two animals died post-surgically (anesthesia), and one animal developed a stereotypy and was eliminated from the study. In the CD condition, six subjects had bilateral lesions of the CE and basal nuclei that were labeled as large (≥½ area at the site of largest damage). In four animals, basal damage included damage to the basomedial and lateral amygdala nuclei. Nine subjects had lesions that were asymmetrical in size. In the ND condition, six subjects had large bilateral lesions of the CE and BLA that included some damage to the BMA. Eight of the subjects had asymmetrical lesions of the CE/BLA area. Twenty-two subjects were in the CD condition for data analysis: Lesion (n = 15) and Control (n = 7); and 21 subjects were in the ND condition for data analysis: Lesion (n = 14) and Control (n = 7). Some damage to the internal capsule, globus pallidus, caudate-putamen, intraamygdaloid bed nucleus of the stria terminalis, interstitial nucleus of the posterior limb of the anterior commissure, anterior, the lateral, cortical and nuclei of the amygdala, amygdalostriatal transition zone, and the ventral and dorsal endipiriform was observed in various subjects but this damage was distributed across groups. In addition, damage to the CE was restricted to mainly the lateral region, leaving most of the medial region intact. Figure 10 provides histological reconstructions of the smallest and largest lesions for the conditioned defeat (left) and no defeat (right) conditions.

Agonistic and Neuroendocrine Responses. The control group was comprised of non-operated and sham controls. There were no differences between these two groups on any of the dependent measures ($p > .05$). Therefore, all data in sham and non-operated lesion control conditions were pooled to form one control condition.

Figure Caption

Figure 10. Histological reconstructions of the smallest (gray/stippled) and largest (black) lesions for the conditioned defeat (left) and no defeat (right) conditions in Experiment 2.



For all dependent measures, homogeneity of variance was tested using Levene's test of homogeneity as in Experiment 1. For submissive/defensive (Day 2) and aggressive (Days 1 and 2) dependent measures, homogeneity of variance was violated ($p < .05$). For these analyses, alpha was set at .01 (Sheskin, 2000).

For Day 1, no significant differences were observed in submissive/defensive ($t_{(20)} = -.707$, $p > .05$), aggressive ($t_{(20)} = 1.334$, $p > .01$), social ($t_{(20)} = .813$, $p > .05$) and nonsocial ($t_{(20)} = .247$), $p > .05$) behavior in response to acute defeat (Figures 11-14). For submissive/defensive behavior on Day 2, a 2 X 2 ANOVA yielded a main effect of amygdala lesion ($F_{(1, 39)} = 26.273$, $p < .01$) and a main effect of condition ($F_{(1, 39)} = 11.001$, $p < .01$). The interaction of lesion and condition was not significant, $F_{(1, 39)} = 4.592$, $p > .01$). Planned comparisons (*t*-tests) indicated that the amount of submissive/defensive behavior shown by lesioned subjects with prior defeat experience was significantly less than that shown by non-lesioned subjects with defeat experience (Figure 15). Also, the amount of submissive/defensive behavior in ND controls appeared less than that of CD controls, though this difference did not reach significance ($p = .06$). Importantly, the amount of aggression displayed by resident aggressors in response to lesioned and non-lesioned subjects was not significantly different, $t_{(20)} = .231$, $p > .05$. (Figure 16).

A 2 X 2 ANOVA revealed a main effect of condition (CD vs. ND) on aggressive behavior ($F_{(1, 39)} = 7.870$, $p < .01$). ND subjects showed significantly more aggressive behavior compared to CD subjects (Figure 17). There were no differences in aggressive behavior due to amygdala lesion ($F_{(1, 39)} = .222$, $p > .01$), and there was no lesion by condition interaction ($F_{(1, 39)} = 1.488$, $p > .01$).

For social behavior, the 2 X 2 ANOVA yielded no lesion ($F_{(1, 39)} = 3.772$, $p > .05$) or condition effects ($F_{(1, 39)} = .038$, $p > .05$), but a significant lesion by condition interaction was found ($F_{(1, 39)} = 5.569$, $p < .05$; Figure 18). Analysis of simple effects for

Figure Caption

Figure 11. Total duration (*M* + *SEM*) of submissive behavior displayed by large (*n* = 15 and control (*n* = 7) amygdala-lesioned animals in response to resident aggressors during a 10-min encounter. No significant differences were found.

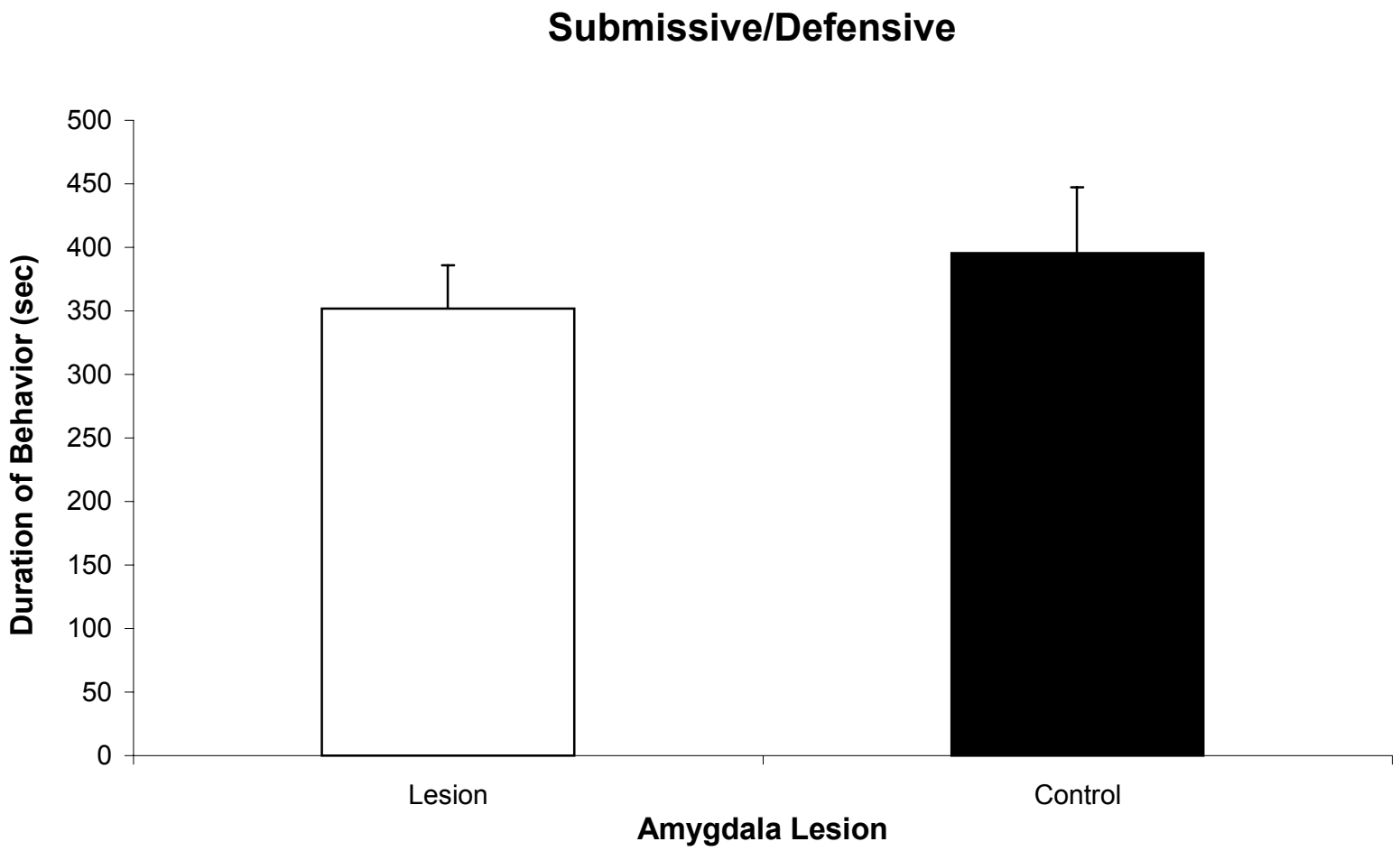

Submissive/Defensive
Duration of Behavior (sec)
500
450
400
350
300
250
200
150
100
50
0
Lesion
Control
Amygdala Lesion

Figure Caption

Figure 12. Total duration (*M* + *SEM*) of aggressive behavior displayed by large (*n* = 15 and control (*n* = 7) amygdala-lesioned animals in response to resident aggressors during a 10-min encounter. No significant differences were found.

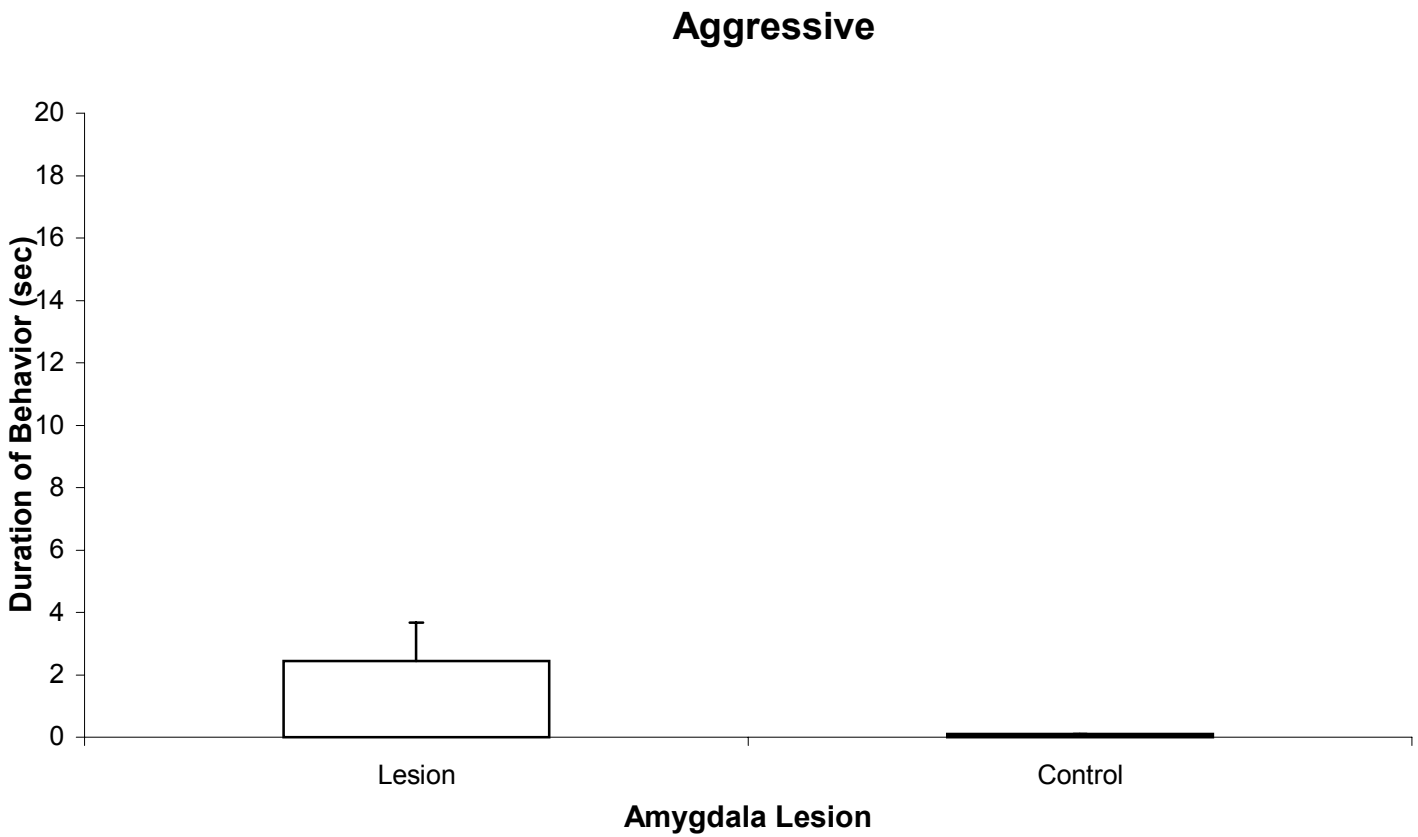

Aggressive
Duration of Behavior (sec)
20
18
16
14
12
10
8
6
4
2
0
Lesion
Control
Amygdala Lesion

Figure Caption

Figure 13. Total duration (*M* + *SEM*) of social behavior displayed by large (*n* = 15 and control (*n* = 7) amygdala-lesioned animals in response to resident aggressors during a 10-min encounter. No significant differences were found.

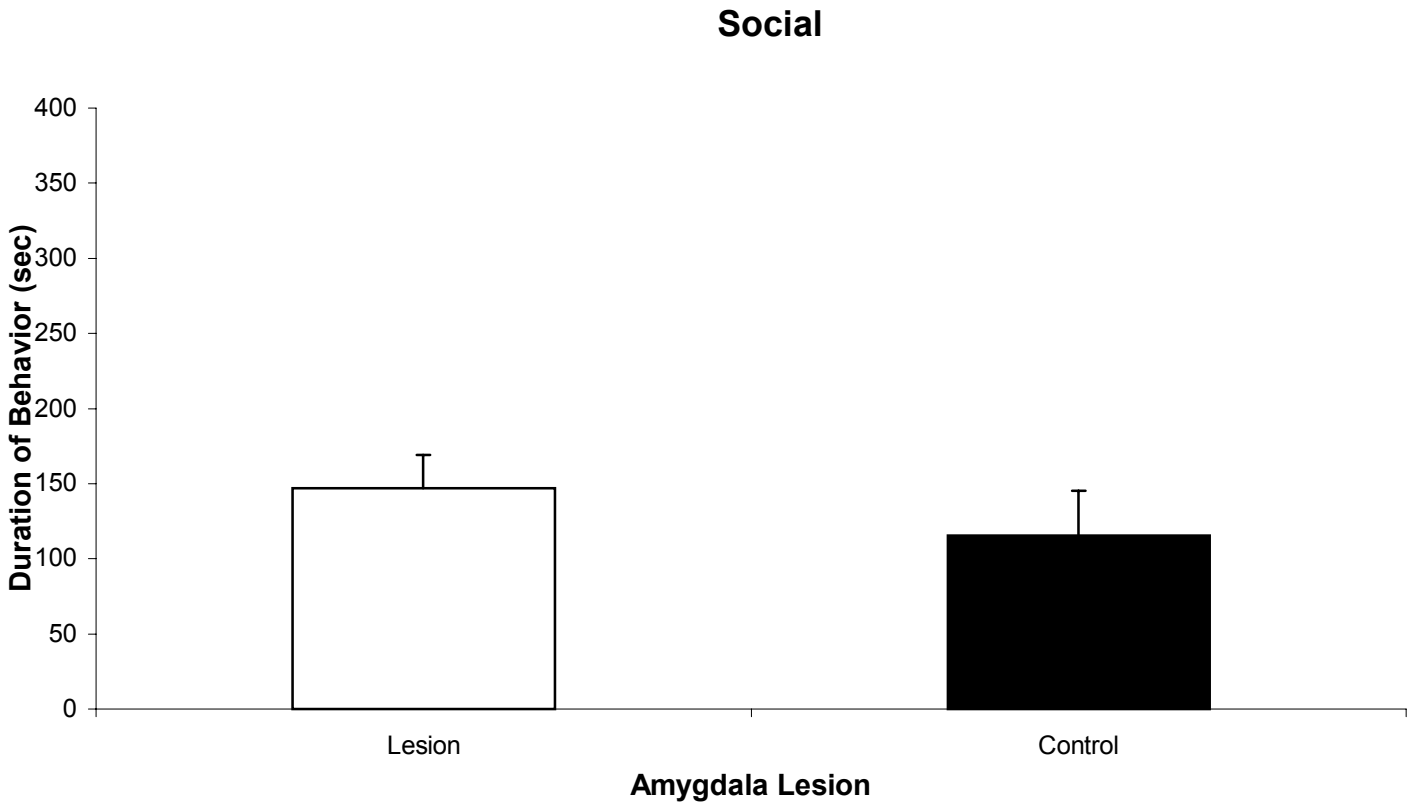

Social
Duration of Behavior (sec)
400
350
300
250
200
150
100
50
0
Lesion
Control
Amygdala Lesion

Figure Caption

Figure 14. Total duration (*M* + *SEM*) of nonsocial behavior displayed by large (*n* = 15 and control (*n* = 7) amygdala-lesioned animals in response to resident aggressors during a 10-min encounter. No significant differences were found.

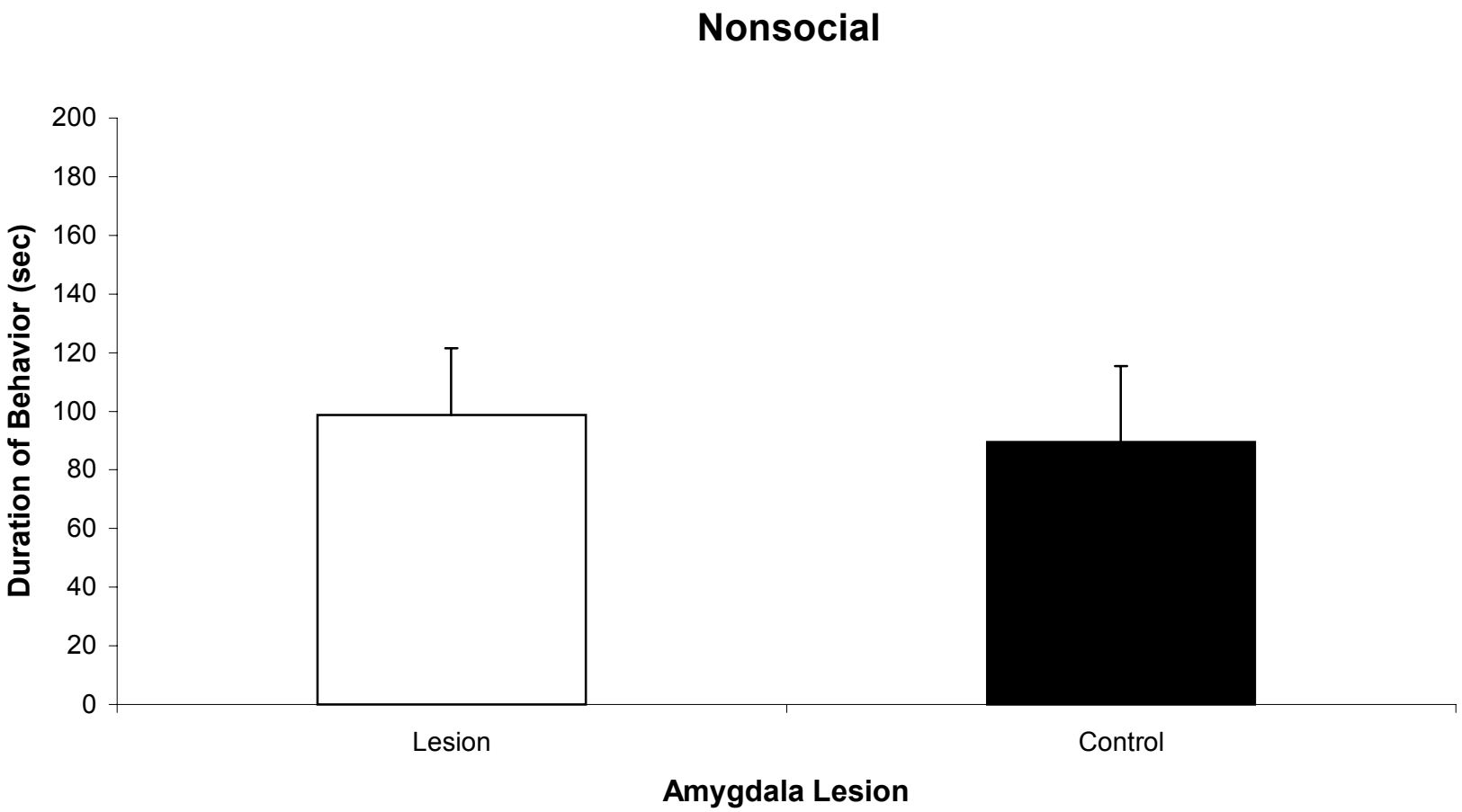

Nonsocial
Duration of Behavior (sec)
200
180
160
140
120
100
80
60
40
20
0
Lesion
Control
Amygdala Lesion

Figure Caption

Figure 15. Total duration (*M* + *SEM*) of submissive behavior displayed by animals with (large [*n* = 15] and control [*n* = 7] amygdala lesions) and without (large [*n* = 14] and control [*n* = 7] amygdala lesions) prior defeat experience in response to a non-aggressive intruder during a 10-min encounter. *Significantly lower than CD lesion control group, $p < .001$.

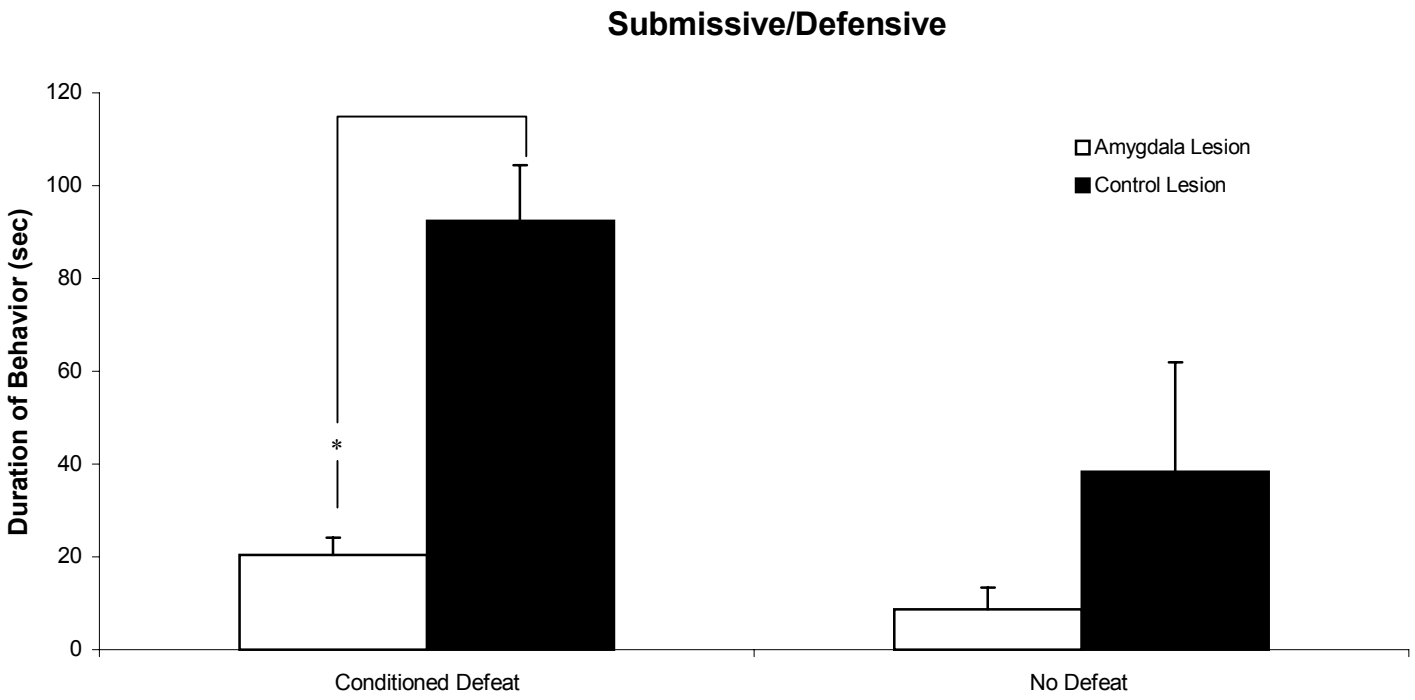

Submissive/Defensive
Duration of Behavior (sec)
120
100
80
60
40
20
0
*
Amygdala Lesion
Control Lesion
Conditioned Defeat
No Defeat

Figure Caption

Figure 16. Total duration (*M* + *SEM*) of aggressive behavior by resident aggressors in response to experimental animals during a 10 min encounter. No significant differences were found.

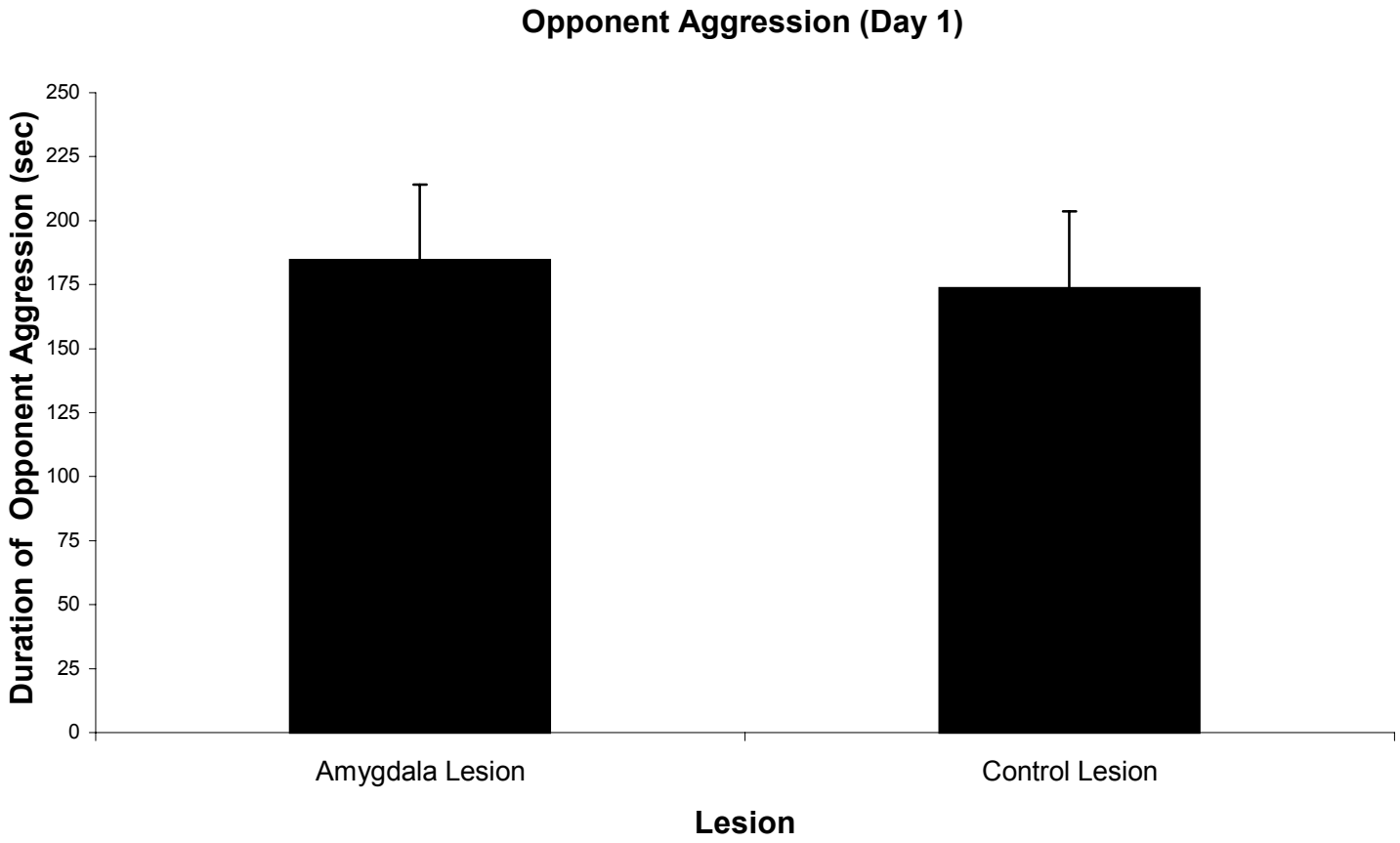

Opponent Aggression (Day 1)
Duration of Opponent Aggression (sec)
250
225
200
175
150
125
100
75
50
25
0
Amygdala Lesion
Control Lesion
Lesion

Figure Caption

Figure 17. Total duration (*M* + *SEM*) of aggressive behavior displayed by animals with (large [*n* = 15] and control [*n* = 7] amygdala lesions) and without (large [*n* = 14] and control [*n* = 7] amygdala lesions) prior defeat experience in response to a non-aggressive intruder during a 10-min encounter. *Significantly lower than No Defeat Condition, $p <$ .01.

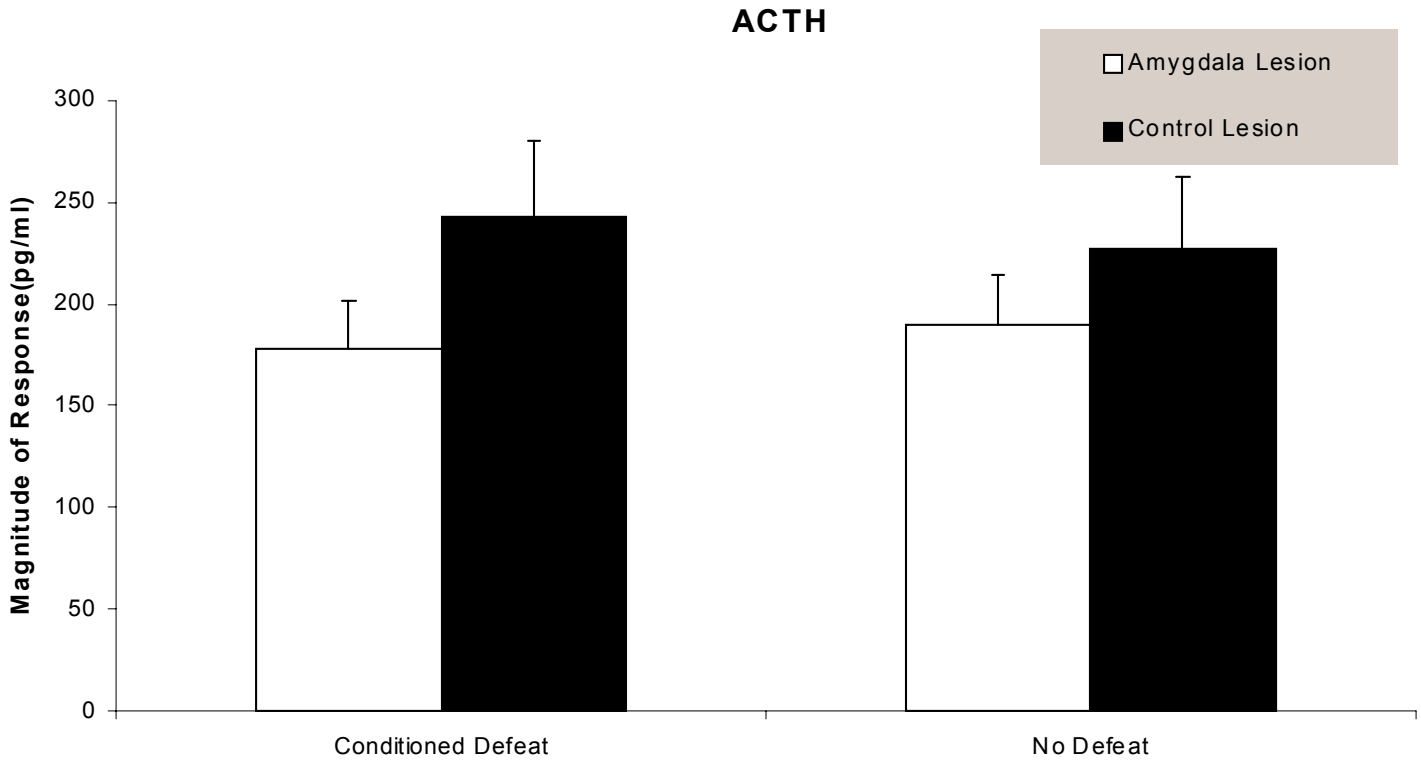
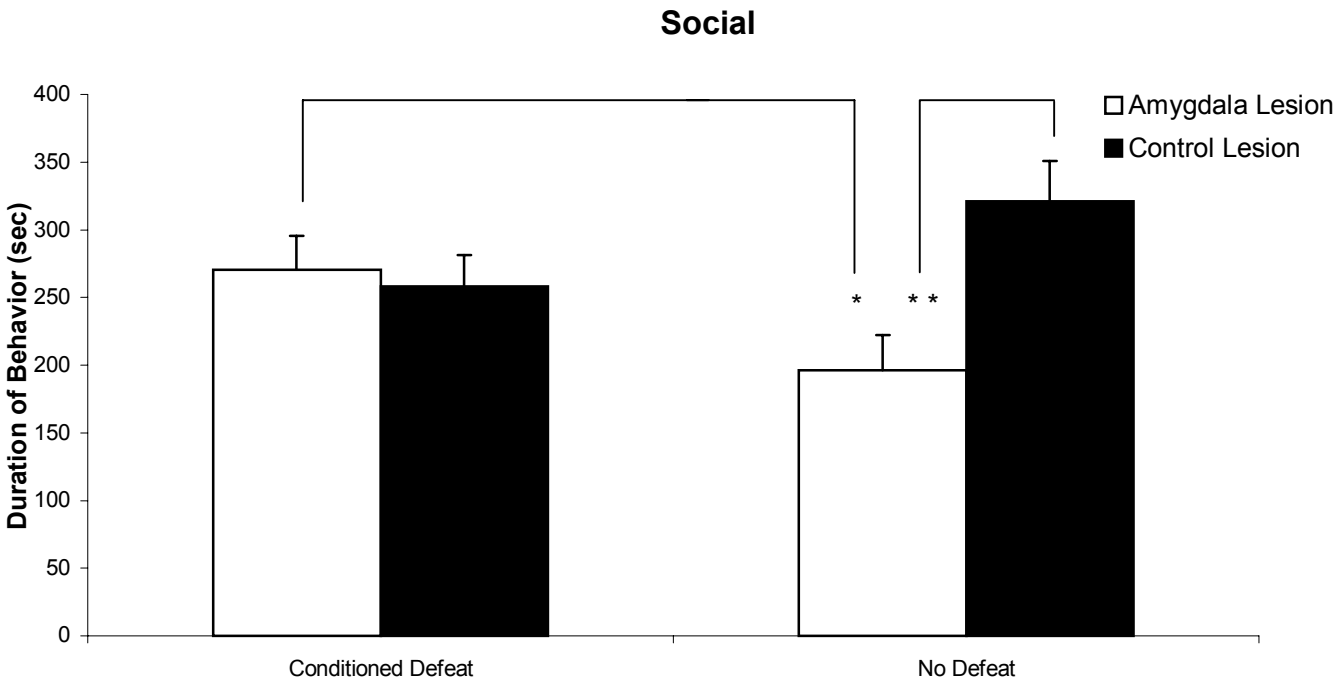

ACTH
Amygdala Lesion
Control Lesion
Magnitude of Response(pg/ml)
300
250
200
150
100
50
0
Conditioned Defeat
No Defeat

Figure Caption

Figure 18. Total duration (*M* + *SEM*) of social behavior displayed by animals with (large [*n* = 15] and control [*n* = 7] amygdala lesions) and without (large [*n* = 14] and control [*n* = 7] amygdala lesions) prior defeat experience in response to a non-aggressive intruder during a 10-min encounter. *Significantly lower than CD lesion group, $p < .05$. **Significantly lower than ND control lesion group, $p < .05$.


Social
Duration of Behavior (sec)
400
350
300
250
200
150
100
50
0
Amygdala Lesion
Control Lesion
*
* *
Conditioned Defeat
No Defeat

the lesion and condition factors yielded two significant simple effects, one at each factor. Lesioned subjects displayed significantly less social behavior in the ND condition compared to the CD condition. Also in the ND condition, subjects with amygdala lesions displayed significantly less social behavior compared to subjects that served as ND controls.

For nonsocial behavior, the 2 X 2 ANOVA revealed a significant main effect of amygdala lesion ($F_{(1, 39)} = 10.963$, $p < .05$), but neither the main effect of condition ($F_{(1, 39)} = .051$, $p > .05$) nor the lesion by condition interaction ($F_{(1, 39)} = 3.188$, $p > .05$) were significant. The Bonferroni-Dunn indicated that nonsocial behavior increased in lesion subjects when compared to controls (Figure 19). Fisher LSD tests (corrected for family-wise error) indicated that this significant difference was due to the potentiation of nonsocial behavior in ND lesions subjects compared to ND controls.

To determine if the increase in nonsocial behavior was due to ataxia or general malaise, a more detailed analysis was conducted. Nonsocial behavior was rescored using locomotor/exploratory, grooming, feeding, sleeping, and nonactivity as behavioral subcategories. Locomotor/exploratory and grooming behavior accounted for 80% and 17% of nonsocial behavior, respectively. Feeding, sleeping, and nonactivity accounting for the remaining 3%. A 2 X 2 ANOVA yielded a significant main effect of lesion on locomotor/exploratory behavior ($F_{(1, 39)} = 18.49$, $p < .05$), but no significant main effect of condition ($F_{(1, 39)} = .46$, $p > .05$) and no significant interaction ($F_{(1, 39)} = 2.28$, $p > .05$) were found. As found in the analysis of nonsocial behavior, post-hoc Fisher LSD tests indicated that this significant difference was due to the potentiation of locomotor/exploratory behavior in ND lesion subjects compared to ND controls (Figure 20). Factorial analyses yielded no significant main effects or interactions for the remaining nonsocial behavior subcategories ($p > .05$).

Figure Caption

Figure 19. Total duration (*M* + *SEM*) of nonsocial behavior displayed by animals with (large [*n* = 15] and control [*n* = 7] amygdala lesions) and without (large [*n* = 14] and control [*n* = 7] amygdala lesions) prior defeat experience in response to a non-aggressive intruder during a 10-min encounter. *Significantly greater than ND control lesion group, $p < .01$.

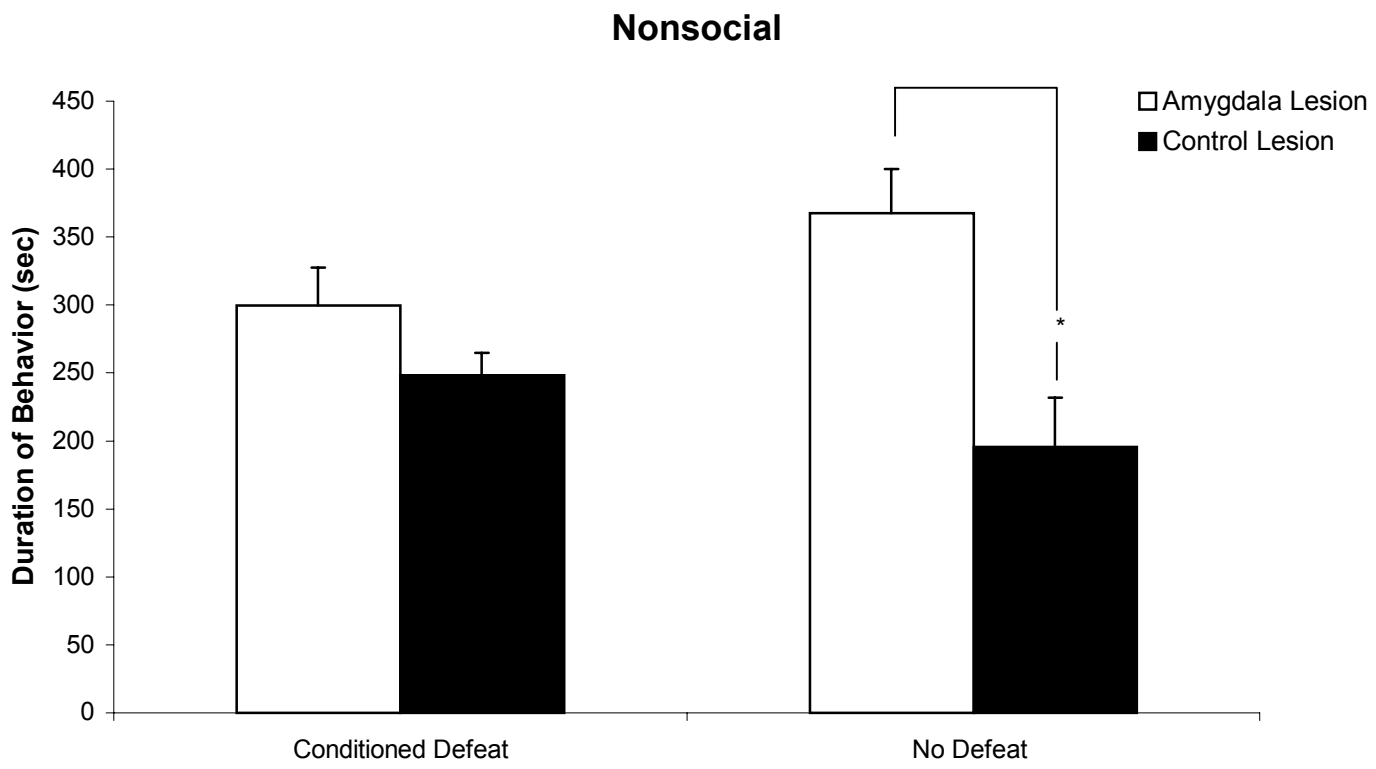
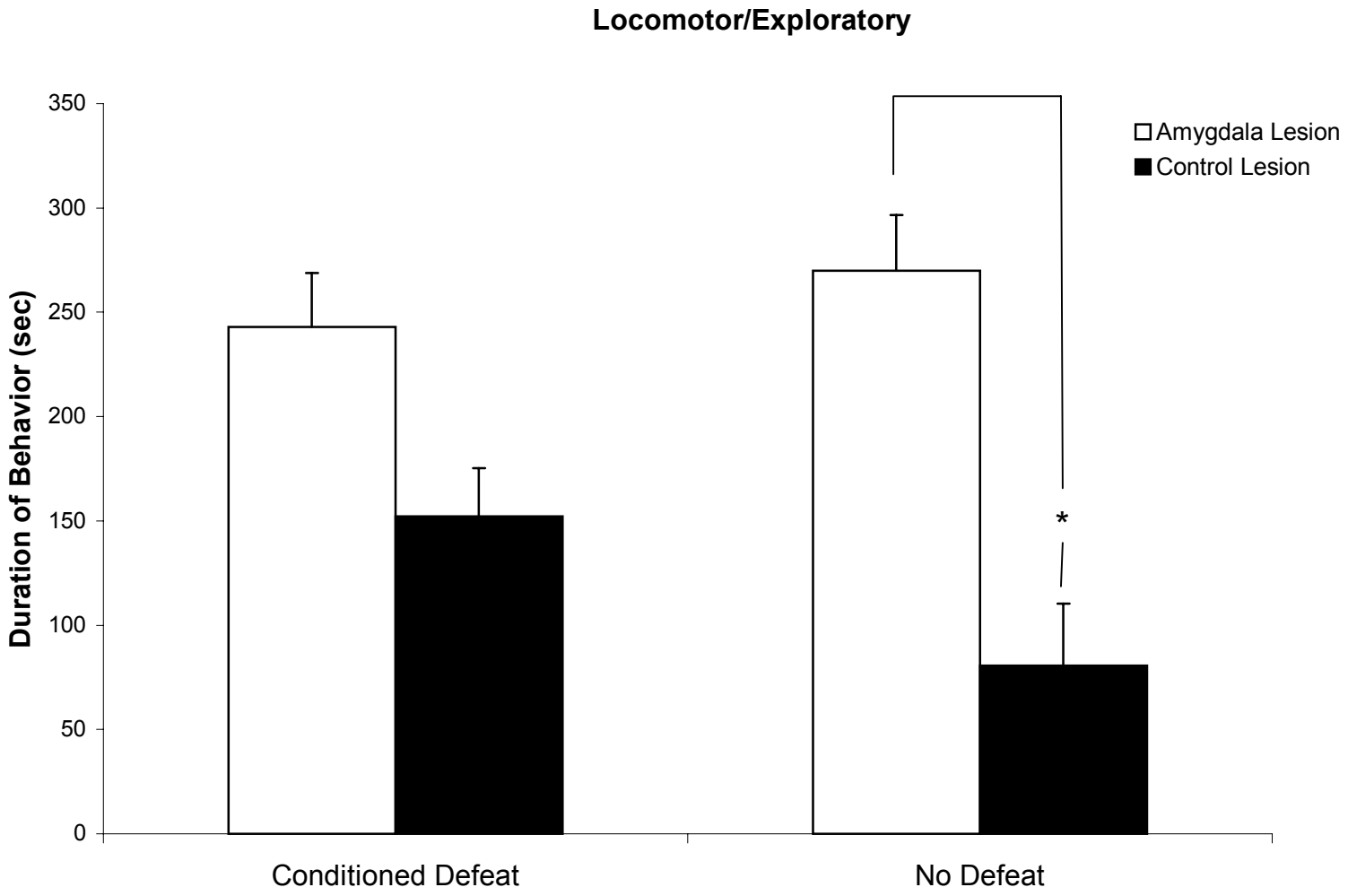

Nonsocial
Amygdala Lesion
Control Lesion
Duration of Behavior (sec)
450
400
350
300
250
200
150
100
50
0
*
Conditioned Defeat
No Defeat

Figure Caption

Figure 20. Total duration (*M* + *SEM*) of locomotor/exploratory behavior displayed by animals with (large [n = 15] and control [n = 7] amygdala lesions) and without (large [n = 14] and control [n = 7] amygdala lesions) prior defeat experience in response to a non-aggressive intruder during a 10-min encounter. *Significantly lower than ND control group, $p < .01$.


Locomotor/Exploratory
Duration of Behavior (sec)
350
300
250
200
150
100
50
0
Amygdala Lesion
Control Lesion
*
Conditioned Defeat
No Defeat

Analyses of hormone data yielded no significant main effects or interactions. For ACTH, a 2 X 2 ANOVA revealed no significant effect of lesion ($F_{(1, 39)} = 2.769.$, $p > .05$), condition, ($F_{(1, 39)} = .003$, $p > .05$) or interaction ($F_{(1, 39)} = .191.$, $p > .05$). A 2 X 2 ANOVA produced similar results for cortisol in that no significant effects were observed due to lesion ($F_{(1, 39)} = .355$, $p > .05$) condition ($F_{(1, 39)} = .419$, $p > .05$) treatment, or interaction ($F_{(1, 39)} = 1.046$, $p > .05$). See Figures 21 and 22 for hormone data.

Figure Caption

Figure 21. Plasma ACTH (*M* + *SEM*) of animals with (large [*n* = 15] and control [*n* = 7] amygdala lesions) and without (large [*n* = 14] and control [*n* = 7] amygdala lesions) prior defeat experience during a 10 min encounter with a non-aggressive intruder. No significant differences were found.

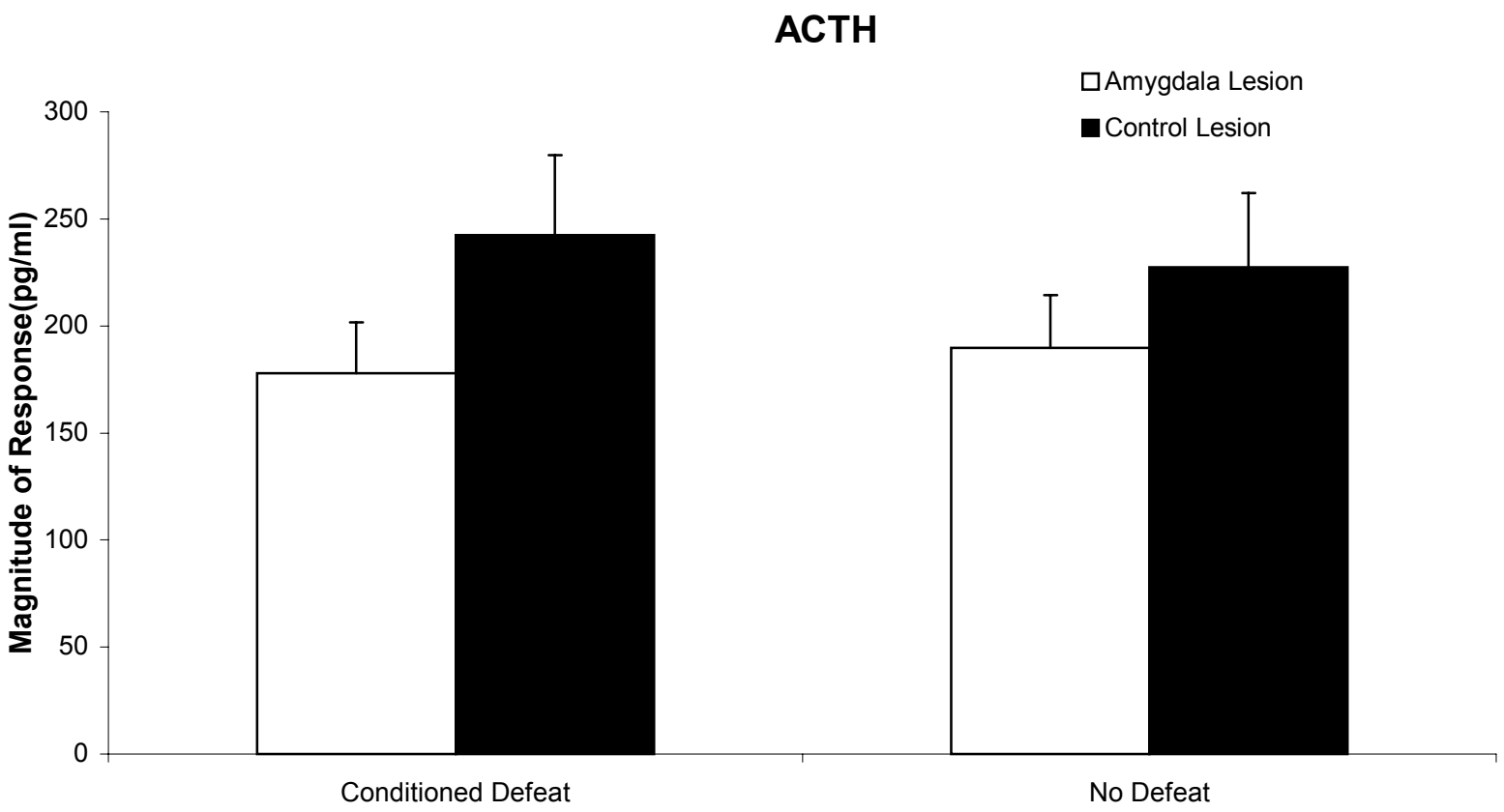
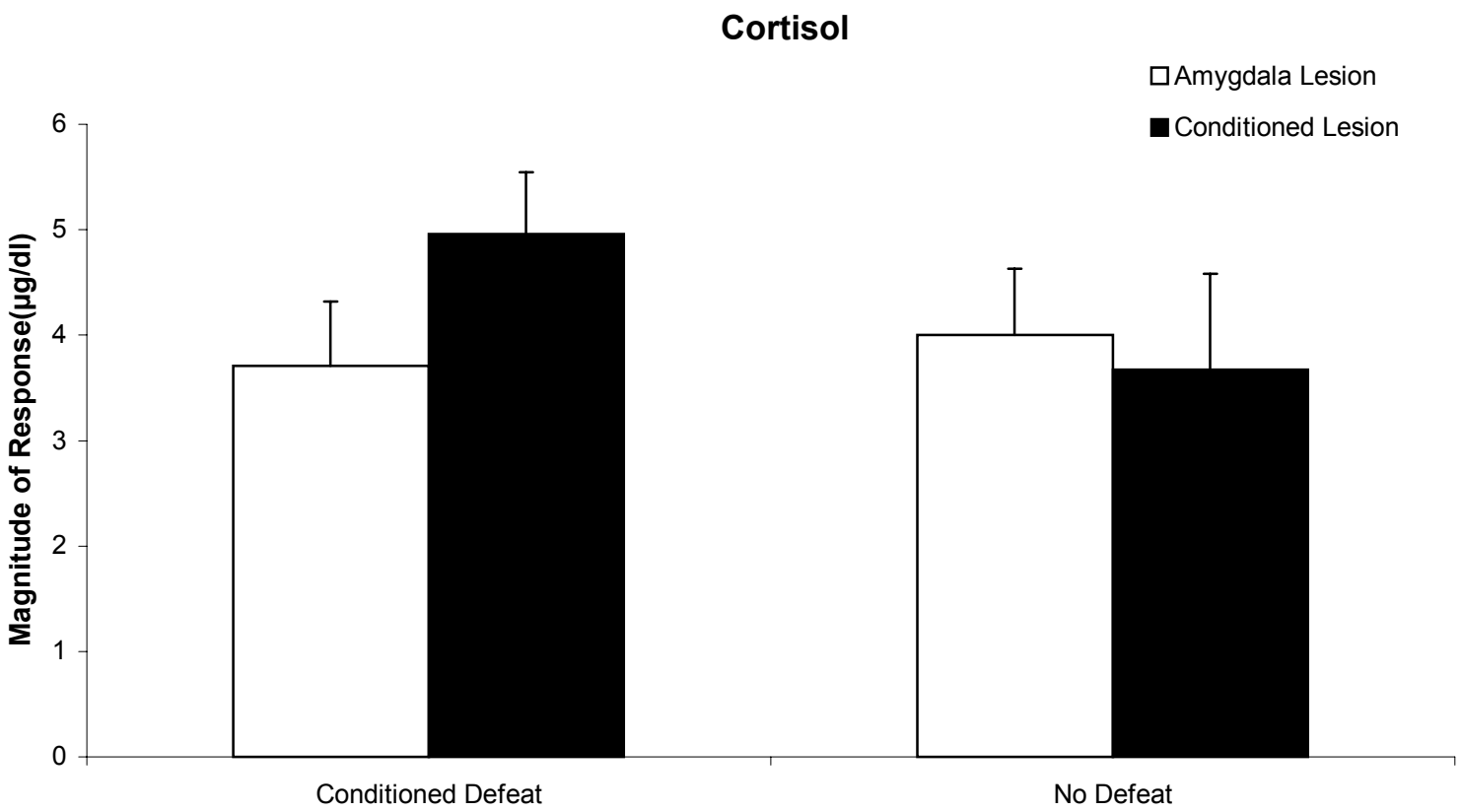

ACTH
Amygdala Lesion
Control Lesion
Magnitude of Response(pg/ml)
300
250
200
150
100
50
0
Conditioned Defeat
No Defeat

Figure Caption

Figure 22. Plasma cortisol (*M* + *SEM*) of animals with (large [*n* = 15] and control [*n* = 7] amygdala lesions) and without (large [*n* = 14] and control [*n* = 7] amygdala lesions) prior defeat experience during a 10 min encounter with a non-aggressive intruder. No significant differences were found.


Cortisol
Amygdala Lesion
Conditioned Lesion
Magnitude of Response(µg/dl)
0
1
2
3
4
5
6
Conditioned Defeat
No Defeat

DISCUSSION

Results from both experiments indicate that large lesions of the amygdala that include the CE and BLA appear to modulate certain agonistic behaviors of both previously-defeated and non-defeated hamsters during an interaction with a non-aggressive intruder without affecting agonistic behavior produced in response to an aggressive opponent. In addition, findings from the present experiments suggest 1) that the CE/BLA amygdala region is not critical in the modulation of neuroendocrine responses of defeat stress and 2) that peripheral ACTH and cortisol levels are independent of the behavioral responses of conditioned defeat. Overall, the results support the hypothesis that the CE/BLA region is involved in the acquisition/expression of conditioned defeat.

Agonistic Behavior

Acute Defeat. In Experiment 1, neither large nor small bilateral lesions of the amygdala affected agonistic behavior (submissive/defensive, aggressive, social, and nonsocial) during a brief encounter with an aggressive opponent. Unilateral lesions did appear to increase aggressive behavior in two of four animals with such lesions, though these subjects also showed high levels of submissive/defensive behaviors. Upon further review of these lesions, no pattern of damage could be associated with this increase in aggressive behavior. Day 1 of Experiment 2 was a replication of Experiment 1, and the lack of lesion effects on agonistic behavior was consistent across these two experiments. On Day 1 of the second experiment, large lesions of the amygdala that included CE and BLA damage had no effect on submissive/defensive, aggressive, social, and nonsocial behavior of an intruder during an initial encounter with an aggressive resident. Specifically, it is important to note that the CE/BLA lesions had in no way blocked the

ability of these animals to produce submissive-defensive behavior because subjects with these lesions were capable of producing normal submissive-defensive behavior in response to an aggressive resident. In sum, the data indicate that the lesioned and non-lesioned animals were exhibiting comparable fear or arousal in response to defeat.

In the present study, the finding that amygdala lesions failed to produce behavioral changes during social conflict failed to support the initial hypothesis but is consistent with studies where amygdala lesions had no effect on agonistic behavior (Blanchard & Takahashi, 1988; Busch & Barfield, 1974; Bolhuis, Fitzgerald, Dijk, & Koolhaas, 1984; Kemble, Blanchard, & Blanchard, 1990; Oakes & Coover, 1996; Shibata, Yamamoto & Ueki, 1982). The results of the present study do appear to contradict Bunnell et al.'s (1970) finding that socially inexperienced hamsters with large amygdaloid lesions were less aggressive and less submissive compared non-lesioned animals. One possibility for this inconsistency may relate to differences in the testing apparatus. Bunnell et al. used a complex cage wherein subjects were allowed to freely enter each other's home cage area and a neutral area that separated the home cages. Differences may also be due to extent of lesion damage. In Bunnell et al.'s (1970) experiment, lesions included damage of the lateral and basolateral amygdala nuclei in the majority of subjects, and damage to medial, basomedial, intercalated, and central amygdala nuclei and the stria terminalis in most subjects. Bolhuis et al. (1994) reported effects similar to those found in the present study in that corticomedial amygdala lesions had no effect on social behavior during defeat. Hence, it is possible that with respect to agonistic behavior, extent of amygdala damage may be important.

Conditioned Defeat. In the conditioned defeat model, the normal aggressive behavior of a resident hamster toward an intruder is replaced by submissive/defensive behaviors in animals that have previously experienced defeat. Because this change in behavior occurs as result of a prior defeat experience, subjects who have not experienced

defeat would be expected to exhibit significantly less submissive/defensive and significantly more aggressive behavior in comparison to previously-defeated subjects. In the present study, ND subjects showed significantly more aggressive behavior than did CD subjects. Overall, very little aggressive behavior was seen in CD subjects (Figure 17) which is consistent with previous findings and with the conditioned defeat model (Jasnow, Banks, Owens, & Huhman, 1999; Jasnow & Huhman, 2001; Potegal et al, 1993). Submissive/defensive behavior in CD controls was not significantly different from that of the of ND control, though Figure 15 does indicate a trend ($p = .06$) for the duration of these behaviors to be greater in the CD controls. This trend may have been rendered nonsignificant by one outlier in the ND control group whose duration of submissive/defensive behaviors was more than two standard deviations above the mean.

In the conditioned defeat model, a significant decrease in the duration of submissive/defensive behaviors and/or the display of aggression in CD subjects denotes a reduction in, or blockade of, conditioned defeat. While prior defeat decreased the amount of aggression a subject displayed toward a non-aggressive intruder, lesion effects were not seen for aggressive behavior. This finding is consistent with studies of the effects of amygdala lesions on aggressive behavior between conspecifics (Blanchard & Takahashi, 1988; Busch & Barfield, 1974; Oakes & Coover, 1996). CE/BLA lesions followed the initial prediction and did significantly decrease submissive/defensive behavior in previously-defeated subjects during an encounter with a non-aggressive intruder. So, these data suggest that CE/BLA lesions reduce the effects of prior defeat on future agonistic behavior. These findings are consistent with recent CD publications (Jasnow, Davis, & Huhman, in preparation; Jasnow & Huhman, 2001). Jasnow and Huhman (2001) have reported that immediate pre-testing infusion of muscimol into the amygdala of previously defeated hamsters attenuates conditioned defeat responses as demonstrated by decreased submissive behavior toward a non-aggressive intruder. Jasnow, Davis, and

Huhman (in preparation) have also reported hamsters with both unilateral CE lesions and a CRH receptor antagonist infused into the bed nucleus of the stria terminalis (BNST) contralateral to the lesion site exhibited significantly less submissive/defensive behavior compared to lesion/drug controls. From these findings it was proposed that CRH originating from cells of the CE acts within the BNST to modulate agonistic responses to social defeat.

In the present investigation, the effects of the amygdala lesions on CD behavior are also consistent with findings of studies that have investigated the effects of pre-training CE lesions on conditioned responses to other aversive stimuli. Subjects with large radio-frequency lesions of the amygdala (including the CE) show attenuated freezing responses to a context previously paired with shock (Blanchard & Blanchard, 1972). Goldstein et al. (1996) reported that pre-training NMDA amygdala lesions (including CE and BLA) block conditioned freezing to tone-context conditioned stimuli. Electrolytic (Oakes & Coover, 1997) and ibotenic acid (Jellestad, Markowaska, Bakke, & Walther, 1986) lesions of the CE have produced deficits in passive avoidance behavior. Pre-training electrolytic (Nader, Majishad, Amorapanth, & LeDoux, 2001) and NMDA (Goosens & Maren, 2001) lesions prevent freezing behavior in an auditory fear conditioning task. Goosens and Maren (2001) also demonstrated that neurotoxin lesions attenuate freezing to contextual stimuli. Recently, Holahan and White (2002) reported that conditioned modulation and conditioned avoidance is impaired following electrolytic lesions of the CE.

It bears mentioning that upon viewing Figure 14 it appears that the amygdala lesions attenuated submissive behavior in both CD and ND conditions. But, a statistically significant decrease in submissive/defensive behavior was seen only in lesioned CD subjects as compared to non-lesioned CD subjects. The pattern of results seems to suggest that the CE/BLA region is involved in the modulation of agonistic

behavior of previously-defeated animals toward non-aggressive intruders. Alternatively, the small amount of submissive/defensive behavior in the ND group may have lead to a floor effect. Future studies could increase contact time with non-aggressive intruders to investigate the possibility that amygdala lesions reduce submissive/defensive behavior in any hamster paired with a non-aggressive opponent.

With respect to social behavior, a significant lesion by condition interaction was found. Specifically, amygdala lesions decreased social behavior in ND lesion subjects as compared to both CD lesion subjects and ND controls (Figure 18). The amount of social behavior in CD lesion, CD control, and ND controls subjects was found to be similar. Therefore, the effect of lesion depended on whether or not the subject had previously experienced defeat. This finding may seem to contradict the results of Experiment 1 and Experiment 2, Day 1. In fact, it may not. One must consider the type of opponent used in each experiment (resident aggressor vs. non-aggressive intruder). It is assumed that non-aggressive intruders are not a direct threat in that they have been treated/chosen in such manner as to substantially decrease the probability of their producing aggressive behavior. It can be speculated that the decrease in social behavior in lesioned, non-defeated animals toward non-aggressive intruders represents a decrease in risk assessment behavior that could not be obtained if the lesioned subject is under direct threat or has had prior experience with defeat.

Nonsocial behavior was increased in lesioned ND subjects as compared to ND controls (Figure 19). To investigate the possibility of this increase in nonsocial behavior being due to ataxia or general malaise, nonsocial behavior was rescored using locomotor/exporatory, grooming, feeding, sleeping, and nonactivity (to indicate possibility of ataxia or general malaise) behavioral subcategories. This increase in nonsocial behavior was almost entirely due to an increase in locomotor/exploratory behavior. This behavior appeared to be normal cage exploration commonly observed

during these tests. Therefore, it is highly unlikely that the increase in nonsocial behavior was due to ataxia, illness or a competing stereotypy. These findings are similar to those seen in Jasnow & Huhman (2001) in which muscimol blocked CD while increasing normal locomotor/exploratory behavior. Because of the way agonistic behavior was scored in their experiment and in the present study (i.e., duration), a decrease in the amount of one or more behavioral categories necessitates an increase in at least one of the other of the categories of behavior.

Neuroendocrine Response

In Experiment 1, there was a condition effect (acute defeat vs. baseline control) for both ACTH and cortisol levels with acute defeat potentiating these neuroendocrine responses above basal levels. This stress response to defeat follows the initial prediction and is consistent with studies that have investigated the neuroendocrine effects of social defeat in male golden hamsters (Huhman et al., 1990, 1991). The lack of a lesion effect on ACTH and cortisol stress responses does not follow the initial prediction and appears to contradict some studies that have reported that CE lesions attenuate neuroendocrine stress responses to acute stressors (Allen & Allen 1974, Roozendaal et al., 1991a; Prewitt & Herman; Van de Kar et al., 1991), though the effects of these lesions, and therefore modulation of these stress responses, may be stressor/modality specific (Allen & Allen, 1974).

In Experiment 2, plasma ACTH and cortisol levels were similar for CD and ND subjects (Figures 19 and 20). Based on the current findings, it appears that regardless of defeat experience and submissiveness, an animal that is paired with a non-aggressive intruder, or NAI, appears to produce a minimal neuroendocrine stress response, thus the baseline levels of ACTH and cortisol were elevated compared to Experiment 1. Interestingly, though interaction with the NAI does not appear to be very stressful, subjects still show submissive behavior. These results do not support initial predictions

and may appear to contradict those of Huhman et al. (1992) who found that the restricted presence of an aggressive resident increased ACTH and cortisol responses in previously defeated hamsters as compared to controls. But, these animals, unlike CD subjects, were paired with their former dominant opponent and were separated from them by a partition that had been removed on previous test days. This difference in context between the two studies may be involved in the inconsistency in stress hormone responsivity subsequent to defeat.

Accordingly, data exist that suggest that defeated hamsters are able to recognize a former opponent. Following defeat by a conspecific, subjects were placed in Y-maze with the winner in an enclosed area at the end of one arm. Compared to males that did not fight, losers of the agonistic encounter took longer to approach their winners and spent most of the time at the base of maze. Losers also spent less time at the end of the arm that contained their winners compared to the amount of time spent in the same area when the winners were not present. Interestingly, males who had not experienced defeat spent most of their time near the winners of previous agonistic encounters and rapidly approached these males. In addition, hamsters spent more time near unfamiliar winners than near familiar winners. These findings suggest that hamsters have the ability to learn to recognize a former opponent that has defeated them (Lai & Johnston, 2002).

CE/BLA lesions had no effect on ACTH and cortisol responses during an encounter with a non-aggressive intruder. On inspection of Figures 21 and 22, the ACTH cortisol responses of lesioned subjects appear to be attenuated, but the differences between lesioned and control subjects were not significant. The present findings contradict studies investigating the role of the amygdala in the neuroendocrine response to conditioned fear (Goldstein et al.,1996, Hebert et al., 1993, Van de Kar et al., 1991, & Roozendaal, et al., 1992). Van de Kar et al. (1991) found that ibotenic lesions of the CE inhibit plasma corticosterone response after exposure to conditioned foot shock stress,

and Roozendaal, et al. (1992) reported that pre-training lesions of the CE and BLA prevent an increase in corticosterone response to a context previously paired with shock. Goldstein et al. (1996) reported that NMDA lesions of the CE block the corticosterone response to contextual cues that were previously paired with shock. Hebert et al. (1993) found that radio-frequency lesions of the CE attenuate plasma ACTH response to contextual cues associated with foot shock.

One explanation for the failure of the CE/BLA lesions to alter plasma levels of ACTH and cortisol during acute defeat is that the CE/BLA may not be directly involved in the neuroendocrine response to acute defeat. In addition, the CE/BLA, while seemingly involved in the modulation of the expression and acquisition of CD behavior, may not be directly involved in the neuroendocrine response to CD testing. Along these lines, the bed nucleus of the stria terminalis (BNST), which is well-positioned to affect the endocrine response to various stressors (Herman & Cullinan, 1997) may play a role in the neuroendocrine response of acute and conditioned defeat. Alternatively, there may have been enough sparing of the CE for the neuroendocrine response to be maintained.

The BNST, considered to be part of the extended amygdala (McDonald, 1992), is a proposed relay center for the convergence of information from the ventral hypothalamus and CE to the paraventricular nucleus of the hypothalamus, or PVN (Gray, Carney, & Magnuson, 1989; Pacak, McCarty, Palkovits, & Goldstein, 1995). The BNST has direct projections to the PVN which contains corticotropin-releasing hormone (CRH) neurosecretory cells. CRH is the main secretagogue of ACTH and is located within the parvicellular cells of the PVN. Accordingly, lateral BNST lesions diminish the ACTH and corticosterone response to a conditioned foot shock stressor (Gray et al., 1989, 1993). Medial BNST lesions increase CRH mRNA expression in the parvocellular region of the PVN, suggesting inhibitory regulation of the PVN (Herman, Cullinan, & Watson, 1994). In anesthetized rats, medial BNST stimulation decreases plasma levels of corticosterone,

while lateral BNST stimulation increases corticosterone levels (Dunn, 1987). Though the involvement of the BNST in the neuroendocrine response to acute defeat may explain the lack of lesion effects in Experiment 1, an alternative explanation may hold true for Experiment 2. For conditioned defeat (Experiment 2) a floor effect may explain the failure of CE/BLA lesions to produce a significant change in the neuroendocrine response of previously-defeated hamsters during an interaction with a non-aggressive intruder. As discussed previously, exposure to a non-aggressive intruder appears to produce a *minimal* stress response.

Interestingly, the neuroendocrine results from Experiment 2 are consistent with previous findings that in certain circumstances behavioral and neuroendocrine responses to social stressors may be disassociated (Jasnow, Banks, Owens, & Huhman, 1999; Heinrichs, Pich, Miczek, Britton, & Koob, 1992; Pich, Heinrichs, Rivier, Miczek, Fisher, & Koob, 1993). Alterations in the behavioral effects of social defeat have been investigated using the elevated plus maze; following defeat, rats spend less time in the exposed arms of the maze (Heinrichs et al., 1992; Pich et al., 1993). Pich et al. found that peripheral application of an anti-CRF serum blocked the ACTH and corticosterone response in rats to social defeat stress. This change in HPA responsivity was not accompanied by changes in the stress-induced behavioral responses in an elevated plus maze. As well, intra-amygdaloid infusions of the CRF receptor antagonist α–helical CRF_{9-41} reversed the defeat stress-induced decrease in time spent in the open arms of the maze, but did not alter the corticosterone and ACTH response to defeat (Heinrichs et al.).

With respect to CD, Jasnow et al. (1999) found that peripheral administration of the CRH_1 receptor antagonist CP-154,526 significantly reduced the plasma level of ACTH in previously-defeated hamsters but failed to alter the expression of conditioned defeat during a subsequent interaction with a non-aggressive intruder. The dissociation of the effects of CE/BLA lesion (current study) and the effects peripheral administration

of CRH antagonist CP-154,526 (Jasnow et al., 1999) do not support to Leshner's (1975) proposal that hormone responses to defeat mediate the effects of the defeat experience on ongoing and future agonistic behavior (Leshner, 1975). Instead, these findings suggest that peripheral levels of ACTH and cortisol do not affect agonistic behavior, at least within the conditioned defeat model.

CONCLUSION

In sum, CE/BLA lesions had no effect on agonistic and neuroendocrine responses to acute defeat, but did reduce the behavioral expression of CD without affecting HPA activity. There is extensive evidence supporting the involvement of the amygdala in fear, anxiety, learning and memory processes. The initial purpose of the current experiment was to investigate the involvement of the CE in the acute and conditioned defeat responses. In this study, damage to both the CE and BLA limits what can inferred concerning their specific involvement in conditioned defeat.

Though studies support the involvement of CE in unconditioned and conditioned fear, extensive data indicate that the BLA could be necessary for performance of unconditioned responses to aversive stimuli (Vazdarjanova, Cahill, & McGaugh, 2001; Wallace & Rosen, 2001), as well as play a role in the acquisition and maintenance of specific CS-US associations(Antoniadis & McDonald, 2001; Maren, 1999; Goosens & Maren, 2001; Vazdarjanova, & McGaugh,1999; Wilensky, Schafe, & LeDoux, 2000) and the expression of conditioned fear (Cousens & Otto, 1998). In addition, the BLA is proposed to be a site of storage of such memories (Fanselow & LeDoux, 1999; Maren, 1999, 2001), though some authors disagree (Cahill, Weinberger, Roozendaal, & McGaugh, 1999). What is generally agreed upon is that the amygdala plays an important role in the behavioral changes that occur in response to aversive stimuli.

Clearer conclusions with respect to the involvement of the amygdala in CD may be made by using more selective lesions (specific nuclei), neurotoxin lesions (sparing most fibers of passage), or pharmacological manipulations (pre-training and post-training manipulations). In fact, Jasnow and Huhman (2001) have shown that immediate pre-testing infusion of muscimol decreased submissive behavior towards a non-aggressive

intruder in animals that were previously defeated. They propose that $GABA_A$ receptors in the CE are important in the acquisition and expression of conditioned defeat. While the present study cannot provide definitive evidence to determine the modulatory role(s) of specific nuclei in conditioned defeat, it does indicate that the CE/BLA region of the amygdala is involved in the modulation of agonistic behavior during an encounter with a non-aggressive intruder. It also follows other recent studies (Jasnow & Huhman, 2001; Jasnow, Davis, & Huhman; in preparation) in suggesting that the amygdala is involved in the behavioral plasticity of conditioned defeat behavior.

REFERENCES

Adams, D. B. (1979). Brain mechanisms for offense, defense, and submission. *The Behavioral and Brain Sciences, 2*, 201-241.

Adams, D. (1986). Ventromedial tegmental lesions abolish offense without disturbing predation or defense. *Physiology and Behavior, 38*(2), 165-168.

Aggleton, J. P. & Mishkin, M. (1986). The amygdala: sensory gateway to the emotions In: Plutchik, R. and Kellerman, H. (Eds.) Emotion: *Theory, Research and Experience*, *Vol. 3*., Orlando, FL Academic Press

Albers, H. E., Yogev, L., Todd, R. B., & Goldman, B. D. (1985). Adrenal corticoids in hamsters: Role in circadian timing. *American Journal of Physiology, 248*, 434-438.

Albert, D. J. & Walsh, M. L. (1984). Neural systems and the inhibitory modulation of agonistic behavior: A comparison of mammalian species. *Neuroscience and Biobehavioral Reviews, 8*, 5-24.

Albonetti, M. E. & Farabollini, F. (1994). Social stress by repeated defeat: Effects on social behaviour and emotionality. *Behavioral Brain Research, 62*, 187-193.

Alheid, G., deOlmos, J. S. & Belramino, C. A. (1995). Amygdala and extended amygdala. In G. T. Paxinos (ed), *The Rat Nervous System,* (pp. 495-578). New York: Academic Press.

Allen, J. P., & Allen C. F. (1974). Role of the amygdaloid complex in the stress induced release of ACTH in the rat. *Neuroendocrinology, 15*, 220-230.

Amat, J., Matus-Amat, P., Watkins, L. R., & Maier, S. F. (1998). Escapable and inescapable stress differentially alter extracellular levels of 5-HT in the basolateral amygdala of the rat. *Brain Research, 812*, 113-120.

Amat, J., Matus-Amat, P., Watkins, L. R., & Maier, S. F. (1998). Escapable and inescapable stress differentially and selectively alter extracellular levels of 5-HT in the ventral hippocampus and dorsal periaqueductal gray of the rat. *Brain Research, 797*, 12-22.

Amoranth, P., LeDoux, J. E., & Nader, K. (2000). Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. *Nature and Neuroscience, 3*, 74-79.

Antoniadis, E. A. & McDonald, R. J. (2000). Amygdala, hippocampus and discriminative fear conditioning to context. *Behavioural Brain Research, 108*, 1-19.

Arahaf, B. M., Kailani, S., & Selman, W. R. (1992). Physiology and pathophysiology of prolactin secretion. In D. Barrow & W. Selman (Eds.), *Neuroendocrinology* (pp. 95-112). Baltimore: Williams & Wilkins.

Asterita, M. F. (1985). The stress response. *The Physiology of Stress* (pp. 35-45). New York: Human Sciences Press.

Axelrod, J. & Reisine, T. D. (1984). Stress hormones: Their interaction and regulation. *Science, 224*, 452-459.

Beaulieu, S., Di Paolo, T. D., & Barden, N. (1986). Control of ACTH secretion by the central nucleus of the amygdala: Implications of the serotonergic system and its relevance to the glucocorticoid delayed negative feedback mechanism. *Neuroendocrinology, 44*, 247-254.

Bennett, B. W., & Whitehead, S. A. (1983). *Mammalian Neuroendocrinology*. New York: Oxford University Press.

Berton, O., Aguerre, S., Sarrieau, A., Mormede, P., & Chaouloff, F. (1998). Differential effects of social stress on central serotonergic activity and emotional reactivity in Lewis and spontaneously hypertensive rats. *Neuroscience, 82,* 147-159.

Black, P. M. (1992). Molecular biology of pituitary hormones. In D. Barrow & W. Selman (Eds.), *Neuroendocrinology* (pp. 65-74). Baltimore: Williams & Wilkins.

Blanchard, D. C., & Blanchard, R., J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. *Journal of Comparative and Physiological Psychology, 81*, 281-290.

Blanchard, D. C. & Blanchard, R. J. (1988). Ethoexperimental approaches to the biology of emotion. *Annual Reviews in Psychology, 39*, 43-68.

Blanchard, D. C., Sakai, R. R., McEwen, B., Weiss, S. M., & Blanchard, R. J. (1993). Subordination stress: Behavioral, brain, and neuroendocrine correlates. *Behavioural Brain Research, 58*, 113-121.

Blanchard, D. C. & Takahashi, S. N. (1988). No change in intermale aggression after amygdala lesions which decrease freezing. *Physiology and Behavior, 42*, 613-616.

Blanchard, R. J, Hebert, M., Sakai, R. R., McKittrick, C., Henrie, A., Yudko, E., McEwen, B. S., & Blanchard, D. C. (1998). Chronic social stress: Changes in behavioral and physiological indices of emotion. *Aggressive Behavior, 24*, 307-321.

Bohus, B., Koolhaas, & J. M. Korte, S. M. (1990). Psychosocial stress, anxiety, and depression: Physiological and neuroendocrine correlated in animal models. In A. R. Genazzani, G. Nappi, F. Petraglia, E. Martignoni (eds), *Stress and related disorders from adaptation to dysfunction*. Pearl City, New York: The Parthenon Publishing Group.

Bolhuis, J. J., Fitzgerald, R. E., Dijk, D. J., & Koolhaas, J. M. (1984). The corticomedial amygdala and learning in an agonistic situation in the rat. *Physiology and Behavior, 32*, 575-579.

Brain, P. F. (1972a). Effects of isolation/grouping on endocrine function and fighting behavior in male and female golden hamsters (*Mesocricetus auratus Waterhouse*). *Behavioral Biology, 7*, 349-357.

Brain, P. F. (1972b). Mammalian behavior and the adrenal cortex—a review. *Behavioral Biology, 7*, 453-477.

Brain, P. F. (1980). Adaptive aspects of hormonal correlates of attack and defense in laboratory mice: a study in ethobiology. *Progress in Brain Research, 53*, 391-413.

Bronson, F., H. (1973). Establishment of social rank among grouped male mice: relative effects on circulating FSH, LH, and corticosterone. *Physiology and Behavior, 10,* 947-951.

Brown, R. E. (1994). *The endocrine glands and their hormones. An introduction to neuroendocrinology* (pp 19-27). London: Cambridge University Press.

Bunnell, B. N., Meyerhoff, J. L., & Kant, G. J. (1988). Psychological stress increases pituitary cyclic AMP. *Pharmacology, Biochemistry,and Behavior, 29*, 151-155.

Bunnell, B. N., Sodetz, F. J., & Shalloway, D. I. (1970). Amygdaloid lesions and social behavior in the golden hamster. *Physiology and Behavior, 5*, 153-161.

Busch, D. E. & Barfield, R. J. (1974). A failure of amygdaloid lesions to alter agonistic behavior in the lab rat. *Physiology and Behavior, 12*, 887-892.

Cahill, L., Wienberger, N., M., Roozendaal, B., & McGaugh, J. L. (1999). Is the amygdala a locus of "conditional fear"? Some questions and caveats. *Neuron, 23*, 227-228.

Campeau, S., & Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *The Journal of Neuroscience, 15*(3), 2301-2311.

Charney, D. S., Grillon, C., & Bremner, D. (1998). The neurobiological basis of anxiety and fear: Circuits, mechanisms, and neurochemical interactions (Part I). *The Neuroscientist, 4*, 35-44.

Chrousos, G. P., Loriaux, L., & Gold, P. (1986). The concept of stress and its historical component. In G. P. Chrousos, L. Loriaux, & P. Gold (Eds.), *Advances in Experimental Medicine and Biology: Mechanisms of Physical and Emotional Stress* (pp. 3-7). New York: Plenum Press.

Cousens, G. & Otto, T. (1998). Both pre-and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behavioral Neuroscience, 112*(5), 1092-1103.

Cullinan, W. E., Herman, J. P., & Watson, S. J. (1993). Ventral subicular interaction with the hypothalamic paraventricular nucleus: Evidence for a relay in bed nucleus of the stria terminalis. *Journal of Comparative Neurology, 332*(1), 1-30.

Davis, M. (1992). The role of the amygdala in conditioned fear. In J. P. Aggleton (Ed.), *The Amygdala: Neurobiological aspects of emotion, memory and mental dysfunction* (pp. 255-305). New York: Wiley-Liss.

Davis, M. (1997). Neurobiology of fear responses: The role of the amygdala. *Journal of Neuropsychiatry, 9*(3), 382-402.

Davis, M. (1998). Are different parts of the extended amygdala involved in fear versus anxiety? *Society of Biological Psychiatry, 44*, 1239-1247.

Davis, M. (2000). In J. P. Aggleton (Ed.), The role of the amygdala in conditioned and unconditioned fear and anxiety. *The Amygdala: A Functional Analysis,* (pp. 289-310). Oxford: Oxford University Press.

Delville, Y., De Vries, G. J., & Ferris, C. F. (2000). Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters. *Brain, Behavior and Evolution, 55*, 53-76.

Delville Y, Melloni R. H. , Jr., & Ferris C. F. (1998). Behavioral and neurobiological consequences of social subjugation during puberty in golden hamsters. *The Journal of Neuroscience, 18,*: 2667-2672.

De Olmos, J. D., Alheid, G. F., & Beltramino, C. A. (1985). Amygdala. In G. T. Paxinos (Ed.), *The rat nervous system: Vol. 1. Forebrain and midbrain.* (pp. 223-334). London: Academic Press.

Deturk, K. H., & Vogel, W. H. (1980). Factors influencing plasma catecholamine levels in rats during immobilization. *Pharmacology Biochemistry & Behavior, 13*, 129-131.

Dunn, J. D. (1987). Plasma corticosterone responses to electrical stimulation of the bed nucleus of the stria terminalis. *Brain Research, 407*, 327-331.

Dunn, J. & Whitener, J., (1986). Plasma corticosterone responses to electrical stimulation of the amygdaloid complex: Cytoarchitectural specificity. *Neuroendocrinology, 42*, 211-217.

Fanselow, M. S. & LeDoux J. E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron, 23*, 229-232.

Feldman, S., Conforti, N., Itzack, A, & Weidenfeld, J. (1994). Differential effect of amygdaloid lesions on CRF-41, ACTH, and corticosterone responses following neural stimuli. *Brain Research, 658*(1-2), 21-26.

Feldman, S., Conforti, N., & Saphier, D. (1990). The preoptic area and bed nucleus of the stria terminalis are involved in the effects of the amygdala on adrenocortical secretion. *Neuroscience, 37*, 775-779.

Fleshner, M., Laudenslager, M. L., Simons, L., & Maier, S. F. (1989). Reduced serum antibodies associated with social defeat in rats. *Physiology and Behavior, 45*: 1183-1187

Flugge, G. (1995). Dynamics of central nervous 5-HT1A-receptors under psychosocial stress. *The Journal of Neuroscience, 15*, 7132-7140.

Frenkel., J. K., Cook, K., Grady, H. J., & S. K. Pendleton. (1965). Effects of hormones on adrenocortical secretion of golden hamsters. *Laboratory Investigations, 14*, 142-156.

Gilbert, M. (1998). Managing stress: Pathways to adaptation and balance. *Health and Nutrition Breakthroughs* [On-line serial]. Available HTPP: www.hnbreakthroughs.com/nov98/stress.cfm.

Goldstein, L. E., Rasmusson, A. M., Bunney, B. S., & Roth, R. H., (1996). Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *The Journal of Neuroscience, 1*6(15), 4787-4798.

Goosens, K. A. & Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learning and Memory, 8*, 148-155.

Gould E., Tanapat, P., Rydel, T., & Hastings, N. (2000). Regulation of hippocampal neurogenesis in adulthood. *Biological Psychiatry, 48*, 715-720.

Grahn, R. E., Will, M. J., Hammack, S. E., Maswood, S., McQueen, M. B., Watkins, L. R., & Maier, S. F. (1999). Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. *Brain Research, 826,* 35-43.

Grant, E. C. & Mackintosh, J. H. (1963). A comparison of the social postures of some common laboratory rodents. *Behaviour, 21*(3-4), 246-259.

Gray, R. S., Carney, M. E., & Magnuson, D. J. (1989). Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus: Possible

role in stress-induced adrenocorticotropin release. *Neuroendocrinology, 50*, 433-446.

Gray, R. S., Piechowski, R. A., Yracheta, J. M., Rittenhouse, R. A., Bethea, C. L., & Van de Kar, L. D. (1993). Ibotenic acid lesions in the bed nucleus of the stria terminalis attenuate conditioned stress-induced increases in prolactin, ACTH, and corticosterone. *Neuroendocrinology, 60*, 517-524.

Greengard, P. & Kebabian, J. W. (1974). Role of cyclic AMP in synaptic transmission in the mammalian peripheral nervous system. *Federation Proceedings, 33*, 1059-1067.

Hall, E. (1992). Amygdaloid structural organization. In B. E. Eleftheriou (Ed.), *Symposium on the neurobiology of the amygdala* (pp. 95-121). New York: Plenum Press.

Hanney, P. W. (1975). Rodents: Their lines and habits. New York: Taplinger.

Hardy, C. A., Quay, J., Livnat, S. & Ader, R. (1990. Altered T-lymphocyte responses following aggressive encounters in mice. *Physiology and Behavior, 47*, 1245-1251.

Hebert, M. A., Bunnell, B. N., Mougey, E. H., & Meyerhoff, J. (1993, November). *Lesions of the central nucleus of the amygdala selectively block hormone responses to psychological stress.* Poster session presented at the annual meeting of the Society for Neuroscience, Washington, D.C.

Hebert, M. A., Potegal, M., Moore, T., Evenson, A. R., & Meyerhoff, J. L. (1996). Diazepam enhances conditioned defeat in hamsters (*Mesocricetus auratus*). *Pharmacology, Biochemistry, and Behavior, 55*, 405-413.

Heinrichs, S. C., Pich, E. M., Miczek, K. A., Britton, K. T., & Koob, G. F. (1992). Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via neurotropic action. *Brain Research, 581*, 190-197.

Helmstetter, F. J. (1992). Contribution of the amygdala to learning and performance of conditional fear. *Physiology and Behavior, 51*, 1271-1276.

Henke, P. G. (1980a). Facilitation and inhibition of gastric pathology after lesions in the amygdala of rats. *Physiology and Behavior, 25*, 575-579.

Henke, P. G. (1980b). The centromedial amygdala and gastric pathology in rats. *Physiology and Behavior, 25*, 107-112.

Henke, P. G. (1982). The telencephalic limbic system and experimental gastric pathology: A review. *Neuroscience and Biobehavioral Reviews, 6*, 381-390.

Henke, P. G. (1985). The amygdala and forced immobilization of rats. *Behavioral Brain Research, 6*, 19-24.

Henke, P. G. (1992). Stomach pathology and the amygdala. In J. P. Aggleton (Ed.), *The Amygdala: Neurobiological aspects of emotion, memory and mental dysfunction* (pp. 323-338). New York: Wiley-Liss.

Herman, J. P. & Cullinan, W. E. (1997). Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. *Neurocircuitry of Stress, 20*(2), 78-84.

Herman, J. P., Cullinan, W. E., & Watson, S. J. (1994). Involvement of the bed nucleus of the stria terminalis in tonic regulation of paraventricular hypothalamic CRH and AVP mRNA expression. *Journal of Neuroendocrinology, 6,* 433-442.

Herman, J. P., Prewitt, C. M. F., & Cullinan, W. E. (1996). Neuronal circuit regulation of the hypothalamo-pituitary-adrenal stress axis. *Critical Reviews in Neurobiology, 10*(3-4), 371-394.

Hitchcock J., & Davis M. (1986). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. *Behavioral Neuroscience, 100*, 11-22.

Holahan, M. R. & White, N. M. (2002). Conditioned memory modulation, freezing, and avoidance as measures of amygdala-mediated conditioned fear. *Neurobiology of Learning and Memory, 77*, 250-275.

Hudgens, G. A. & MacNeil, D. A. (1970). Aggressiveness and learning ability: Effects of histories of wins or defeats in avoidance learning in mice. *Psychonometric Science, 20*(1), 51-53.

Huhman, K. L., Bunnell, B. N., Mougey, E. H., & Meyerhoff, J. L. (1990). Effects of social conflict on POMC-derived peptides and glucocorticoids in male golden hamsters. *Physiology and Behavior, 47*, 949-956.

Huhman, K. L., Moore, T. O., Ferris, C. F., Mougey, E. H., & Meyerhoff, J. L. (1991). Acute and repeated exposure to social conflict in male golden hamsters: Increases in plasma POMC-peptides and cortisol and decreases in plasma testosterone. *Hormones and Behavior, 25*, 206-216.

Huhman, K. L., Moore, T. O, Mougey, E. H., & Meyerhoff, J. L. (1992). Hormonal responses to fighting in hamsters: Separation of physical and psychological causes. *Physiology and Behavior, 51*, 1083-1086.

Huhman, K. L., Mougey, E. H., Moore, T. O, & Meyerhoff, J. L. (1995). Stressors, including social conflict, decrease plasma prolactin in male golden hamsters. *Hormones and Behavior, 29*, 581-592.

Isaacson, R. L. (1982a). The structure of the limbic system. *The Limbic System* (pp. 1-60). New York: Plenum Press.

Isaacson, R. L. (1982b). The graven image lethe and the guru. *The Limbic System* (pp. 239-257). New York: Plenum Press.

Isaacson, R. L. (1993). A "fuzzy set" perspective of the limbic system: Implications for cognition and memory. *Neuroscience Research Communications, 13*(1), S15-S18

Jasnow, A. M., Banks, M. C., Owens, E. C., & Huhman, K. L. (1999). Differential effects of two corticotropin-releasing factor antagonists on conditioned defeat in male Syrian hamsters (*Mesocricetus auratus*). *Brain Research*, *846*, 122-128.

Jasnow, A. M., Davis, M., & Huhman, K. L. (in preparation). Corticotropin-releasing hormone receptors modulate conditioned defeat within the bed nucleus of the stria terminalis, but not the amygdala.

Jasnow, A. M., Drazen, D. L., Huhman, K. L., Nelson, R. J., & Demas, G. E. (2001). Acute and chronic social defeat suppresses humoral immunity of male Syrian hamsters (*Mesocricetus auratus*). *Hormones and Behavior, 40*, 428-433

Jasnow, A. M. & Huhman, K. L. (2001). Activation of $GABA_A$ receptors in the amygdala blocks the acquisition and expression of conditioned defeat in Syrian hamsters. *Brain Research, 920*(1-2), 142-150.

Jellestad, F. K., Markowska, A., Bakke, H. K., & Walther, B. (1986). Behavioral effects after ibotenic acid, 6-OHDA, and electrolytic lesions in the central amygdala nucleus of the rat. *Physiology and Behavior, 37*, 855-862.

Kant, G. J., Meyerhoff, J. L., Bunnell, B. N., & Lenox, R. H. (1982). Cyclic AMP and cyclic GMP response to stress in brain and pituitary: Stress elevates pituitary cyclic AMP. *Pharmacology, Biochemistry, and Behavior, 17*, 1067-1072.

Kapp, B. S., Frysinger, R. C., Gallagher, M., & Haselton, J. R., (1979). Amygdaloid central nucleus: Effect on heart rate conditioning in the rabbit. *Physiology and Behavior, 23*, 1109-1117.

Kapp, B. S., Gallagher, M., Frysinger, R. C., & Applegate, C. D. (1981). The amygdala, emotion, and cardiovascular conditioning. In Y. Ben-Ari (Ed.) *The Amygdaloid complex: INSERM symposium No. 20* (pp.355-366). New York: Elsevier.

Kemble, E. D., Blanchard, D. C., & Blanchard R. J. (1990). Effects of regional amygdaloid lesions on flight and defensive behaviors of wild black rats (*Rattus rattus*). *Physiology and Behavior, 48,* 1-5.

Kemble, E. D., Blanchard, D. C., Blanchard R. J. & Takahashi, S. N. (1984). Taming in wild rats following medial amygdaloid lesions. *Physiology and Behavior, 32*, 131-134.

Kim M, Campeau S, Falls WA, Davis, M. (1993). Infusion of the non-NMDA receptor antagonist CNQX into the amygdala blocks the expression of fear-potentiated startle. *Behavior and Neural Biology, 59*, 5-8.

Kim, M., & Davis, M. (1993). Electrolytic lesions of the amygdala block acquisition and expression of fear-potentiated startle even with extensive training but do not prevent reacquisition. *Behavioral Neuroscience 107,* 580-595.

Kim, J. J.. Rison, R. A., & Fanselow, M. S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behavioral Neuroscience, 107*, 1093-98.

Kling, A. S. & Brothers, L. A. (1992). Amygdala and social behavior. In J. P. Aggleton (Ed.), *The Amygdala: Neurobiological aspects of emotion, memory and mental dysfunction* (pp. 353-377). New York: Wiley-Liss.

Klüver, H. & Bucy, P. C. (1937). "Psychic blindness" and other symptoms following bilateral temporal lobectomy in rhesus monkeys. *American Journal of Physiology, 119*, 352-353.

Kolb, B., & Nonneman, A. J. (1973). Frontolimbic lesions and social behavior in the rat. *Physiology and Behavior, 13*, 637-643.

Kollack-Walker, S., Watson, S. J., & Akil, H. (1997). Social stress in hamsters: Defeat activates specific neurocircuits within the brain. *The Journal of Neuroscience, 17*(22), 8842-8855.

Kollack-Walker, S., Don, C., Watson, S. J., & Akil, H. (1999). Differential expression of c–*fos* mRNA within Neurocircuits of male hamsters exposed to acute or chronic defeat. *Journal of Neuroendocrinology, 11*, 547-559.

Kopin, I. J., Eisenhofer, G., & Goldstein, D. (1986). Sympathoadrenal medullary system and stress. In G. P. Chrousos, L. Loriaux, & P. Gold (Eds.), *Advances in experimental medicine and biology: Mechanisms of physical and emotional stress* (pp. 11-23). New York: Plenum Press.

Lai, W., & Johnston, R. E. (2002). Individual recognition after fighting by golden hamster: A new method. *Physiology and Behavior*, 76, 225-239.

Landau, I. T. (1975). Light-dark rhythms in aggressive behavior of the male golden hamster. *Physiology and Behavior, 14*, 767-774.

LeDoux, J. (2000). In J. P. Aggleton (Ed.), The amygdala and emotion: A view through fear. *The Amygdala: A Functional Analysis* (2nd edition; pp. 289-310). Oxford: Oxford University Press.

LeDoux, J. E., Iwata, J., Cicchetti, P. & Reis, D. J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *The Journal of Neuroscience, 8*(7), 2517-2529.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The lateral amygdala nucleus: Sensory interface of the amygdala in fear conditioning. *The Journal of Neuroscience, 10*, 1062-1069.

Lee, G. P., Arena, J. G., Meador, K. J., Smith, J. R., Loring, D. W., & Flanigin, H. F. (1988). Change in autonomic responsiveness following bilateral amygdalotomy in man. *Neuropsychiatry, Neurophysiology, and Behavioral Neurology, 1*, 119-129.

Lee, Y. & Davis, M. (1997). Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing

hormone on the acoustic startle reflex. *The Journal of Neuroscience, 17*(16), 6434-6446.

Lerwill, C. J. , & Makings, P. (1971). The agonistic behavior of the golden hamster *Mesocriatus auratus* (Waterhouse). *Animal Behavior, 19*, 714-721.

Leshner, A. (1975). A model of hormones and agonistic behavior. *Physiology and Behavior, 15*, 225-235.

Leshner, A. I. & Politch, J. A. (1979). Hormonal control of submissiveness in mice: Irrelevance of the androgens and relevance of the pituitary-adrenal hormones. *Physiology and Behavior, 22*, 531-534.

Luciano, D., S., Vander, A. J., & Sherman, J. H. (1983). *Human Anatomy and Physiology*. New York McGraw-Hill.

Makara, G. B. (1985). Mechanisms by which stressful stimuli activate the pituitary-adrenal system. *Federation Proceedings, 44*(1, Pt 2), 149-153.

Marcilhac, A. & Siaud, P. (1996). Regulation of the adrenocorticotrophin response to stress by the central nucleus of the amygdala in rats depends upon the nature of the stressor. *Experimental Physiology, 81*, 1035-1038.

Maren, S. (1999). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *The Journal of Neuroscience, 19*(19), 869-8703.

Maren., S. (2001). Is there a savings for Pavlovian fear conditioning after neurotoxic basolateral amygdala lesions in rats*? Neurobiology of Learning and Memory, 76*, 268-283.

Maren, S. & Fanselow, M. S. (1996). The amygdala and fear conditioning: Has the nut been cracked? *Neuron, 16*, 237-240.

Martinez, M., Calvo-Torrent, A., & Pico-Alfonso, M. A. (1998). Social defeat and subordination as models of social stress in laboratory rodents: A review. *Aggressive Behavior, 24*, 241-256.

Mason, J. W. (1975). A historical view of the stress field. *Journal of Human Stress*, *1*(2), 22-36.

Maswood, S., Barter, J. E., Watkins, L. R., & Maier, S. F. (1998). Exposure to inescapable but not escapable shock increases extracellular levels of 5-HT in the dorsal raphe nucleus of the rat. *Brain Research, 783*, 115-120.

Matheson, G. K. Branch, B. J., & Taylor, A. N. (1971). Effects of amygdaloid stimulation on pituitary-adrenal activity in conscious cats. *Brain Research, 32*, 151-167.

Meerlo, P., Overkamp, G. J., Daan S., Van Den Hoofdakker, R. H., & Koolhaas, J. M. (1996a). Changes in behaviour and body weight following a single or double social defeat in rats. *Stress, 1*, 21-32

Meerlo, P., Overkamp, G. J., Daan S., Van Den Hoofdakker, R. H., & Koolhaas, J. M. (1996b). Long-term changes in open field behaviour following a single social defeat in rats can be reversed by sleep deprivation. *Physiology and Behavior, 60*, 115-119.

McCann, S. M. (1988). The anterior pituitary and hypothalamus. In J. E. Griffin & S. R. Ojeda (Eds.), *Textbook of Endocrine Physiology* (pp. 70-99). New York: Oxford University Press.

McCarty, R. & Kopin, I. (1979). Stress-induced alteration in plasma catecholamines and behavior of rats: Effects of chlorisondamine and bretylium. *Behavioral and Neural Biology, 27,* 249-265.

McDonald, A. J. (1992). Projection neurons of the basolateral amygdala: A correlative Golgi and retrograde tract tracing study. *Brain Research Bulletin, 28*: 179-185.

McKittrick, C. R., Blanchard, D. C., Blanchard, R. J., McEwen, B. S., & Sakai, R. R. (1995). Serotonin receptor binding in a colony model of chronic social stress. *Biological Psychiatry, 37*, 383-393.

Meyerhoff, J. L., Oleshanshy, M. A., & Mougey, M. S. (1988). Psychological stress increases plasma levels of prolactin, cortisol, and POMC-derived peptides in man. *Psychosomatic Medicine, 50*, 286-294.

Moberg, G. P. (1985). Biological Responses to Stress: Key to assessment of animal well-being? In G. P. Moberg (Ed.), *Animal Stress* (pp. 27-59). Baltimore: Waverly Press.

Morin, L. P. & Wood, R. J. (2001). *Stereotaxic atlas of the golden hamster brain.* San Diego, CA: Academic Press.

Nader, K., Majidishad, P., Amorapanth, P., & LeDoux, J. E. (2001). Damage to lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. *Learning and Memory, 8*, 156-163.

Natelson, B. H., Creighton, D., McCarty, R., Tapp, W. N., Pitman, D. & Ottenweller, J. E. (1987). Adrenal hormonal indices of stress in laboratory rats. *Physiology and Behavior, 39,* 117-125.

Natelson, B. H., Holaday, J., Meyerhoff, J., & Stokes, P. E. (1975). Temporal changes in growth hormone, cortisol, and glucose: Relation to light onset and behavior. *American Journal of Physiology, 229*(2), 409-415.

Natelson, B. H., Tapp, W. N., Adamus, J. E., Mittler, J. C., & Levin, B. E. (1981). Humoral indices of stress in rats. *Physiology and Behavior, 26*, 1049-1054.

Nock, B. L., & Leshner, A. I. (1976). Hormonal mediation of the effects of defeat on agonistic responding in mice. *Physiology and Behavior, 17*, 111-119.

Oakes, M. E. & Coover, G. P. (1997). Effects of small amygdala lesions on fear, but not aggression, in the rat. *Physiology and Behavior, 61*(1), 45-55.

Ottenweller, J. E., Tapp, W. N., Burke, J. M., & Natelson, B. H. (1985). Plasma cortisol and corticosterone concentrations in the golden hamster, (*Mesocricetus auratus*). *Life Sciences, 37*, 1551-1558.

Pacak, K., McCarty, R., Palkovits, M., Kopin, I. J., & Goldstein, D. S. (1995). Effects of immobilization on in vivo release of norepinephrine in the bed nucleus of the stria terminalis in conscious rats. *Brain Research, 688*, 242-246.

Pich, E. M., Heinrichs, S. C., Rivier, C., Miczek, A. K., Fisher, D. A., & Koob, G. F. (1993). Blockade of pituitary-adrenal axis activation by peripheral immunoneutralization of corticotropin-releasing factor does not affect the behavioral response to social defeat stress in rats. *Psychoneuroendocrinology, 18*, 495-507.

Pollock, K., (1988). On the nature of social stress: Production of a modern mythology. *Social Science Medicine, 26*(3), 381-392.

Politch, J. A., & Leshner, A. L. (1977). Relationship between plasma corticosterone levels and levels of aggressiveness in mice. *Physiology and Behavior, 19*, 775-780.

Potegal, M., Huhman, K., Moore, T., & Meyerhoff, J. (1993). Conditioned defeat in the Syrian golden hamster (*Mesocricetus auratus*). *Behavioral and Neural Biology, 60*, 93-102.

Prewitt, C. M. & Herman. J. P. (1994). Lesion of the central nucleus of the amygdala decreases basal CRH mRNA expression and stress-induced ACTH release. In R. de Kloet, E. C. Azmitia, & P. W. Landfield (Eds.), *Brain corticosteroid receptors* (pp. 438-440). New York: The New York Academy of Sciences.

Reichlin, S. (1992). Neuroendocrinology. In J. D. Wilson & D. W. Foster (Eds.), *William's Textbook of Endocrinology* (pp. 135-199). Philadelphia: Saunders.

Rook, J. C. (1973). *Localizing the hoarding deficit in the septal hamster.* Unpublished doctoral dissertation, University of Georgia, Athens.

Roozendaal, B., Koolhaas, J. M., & Bohus, B. (1990). Differential effect of lesioning of the central amygdala on the bradycardiac and behavioral response of the rat in relation to conditioned social and solitary stress. *Behavioral Brain Research, 41*, 39-48.

Roozendaal, B., Koolhaas, J. M., & Bohus, B. (1991a). Attenuated cardiovascular, neuroendocrine, and behavioral responses after a single footshock in central amygdaloid lesioned male rats. *Physiology and Behavior, 50*, 771-775.

Roozendaal, B., Koolhaas, J. M., & Bohus, B. (1991b). Central amygdaloid lesions affect behavioral and autonomic balance during stress in rats. *Physiology and Behavior, 50*, 777-781.

Roozendaal, B., Koolhaas, J. M., & Bohus, B. (1992). Central amygdaloid involvement in neuroendocrine correlates of conditioned stress responses. *Journal of Neuroendocrinology, 4*, 483- 489.

Sananes, C. B., Davis, M. (1992). N-methyl D-aspartate lesions of the lateral and basolateral nuclei of the amygdala block fear-potentiated startle and shock sensitization of startle. *Behavioral Neuroscience, 106*, 72-80.

Sapolsky. R. M. (1992). Neuroendocrinology of the stress response. In J. B. Becker, S. M. Breedlove, & D. Crews (Eds.). *Behavioral Endocrinology* (pp. 287-324). Cambridge, MA: MIT Press.

Sapolsky, R. M., Uno, H. Rebert, . & Finch, C. E. (1990). Hippocampal damage associated with prolonged glucocorticoid exposure in primates. *The Journal of Neuroscience, 7*, 3198-3206.

Schuurman, T. (1980). Hormonal correlates of agonistic behavior in adult male rats. *Progress in Brain Research*, 53, 415-420.

Schindler, W. J. & Knigge, K. M. (1959). Adrenal cortical secretion by the golden hamster. *Endocrinology, 65*, 739-747.

Seggie, J. (1979). Differential effects of basolateral amygdala lesions on behavior, corticosterone, and prolactin responses. *Psychiatry Research, 1*, 283-295.

Seggie. J. (1980). Amygdala lesions and 24-h variation in plasma corticosterone, growth hormone, and prolactin levels. *Canadian Journal of Physiology and Pharmacology, 58*, 249-253.

Seggie, J. (1983) Corticomedial amygdala lesions, behavior, corticosterone, and prolactin: Unexpected separation of effects. *Psychiatry Research, 10*, 139-150.

Seggie, J. & Uhlir, I. (1979). Parallel changes in behavior and plasma levels of hormones in septal rats. *Psychoneuroendocrinology, 4*, 135-144.

Seggie, J., Uhlir, I., & Brown, G. M. (1974). Adrenal stress responses following septal lesions in the rat. *Neuroendocrinology, 16*, 225-236.

Selye, H. (1975). Confusion and controversy in the stress field. *The Journal of Human Stress, 1,* 37-44.

Selye, H. (1976a). My First Glimpse of Stress. *The Stress of Life* (pp. 15-28). New York: McGraw Hill.

Selye, H. (1976b). The Birth of the G.A.S. *The Stress of Life* (pp. 29-54). New York: McGraw Hill.

Seward. J. P. (1946). Aggressive behavior in the rat. *Journal of Comparative Psychology, 39*, 51-76.

Serova, L. I., Bozlova, O. N., Rivkin, M. I., & Naumenko, E. V. (1992). Tyrosine hydroxylase activity and gene expression in mice with contrasting dominating power during social stress. *Bulletin of Experimental Biology and Medicine, 113,* 108-110.

Sgoifo, A., de Boer, S. F., Haller, J. & Koolhaas, J. M. (1996). Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: Relationships with aggression. *Physiology and Behavior, 60*, 1403-1407.

Shepard, G. M. (1994). Emotion. In *Neurobiology,* (pp. 603-617). New York: Oxford University Press.

Sheskin, D. J. (1997). *Handbook of parametric and nonparametric statistical procedures*. Boca Raton, FL: CRC Press.

Shibata, S. & Yamamoto, T. & Ueki, S. (1982). Differential effects of medial, central, basolateral amygdaloid lesions on four models of experimentally-induced aggression in rats, *Physiology and Behavior, 28*, 289-294.

Shipley, J. E. & Kolb, B. (1977). Neural correlates of species-typical behaviors in the Syrian golden hamster. *Journal of Comparative Physiological Psychology, 91*, 1056-1073.

Siegel, A. & Edinger, H. M. (1983). Role of the limbic system in hypothalamically elicited attack behavior. *Neuroscience and Biobehavioral Reviews, 7*, 395-407.

Siegel, A., Roeling, T. A., Gregg, T. R. & Kruk, M. R. (1999). Neuropharmacology of brain-stimulation-evoked aggression. *Neuroscience and Biobehavioral Reviews, 23*, 359-389.

Sodetz, F. J. & Bunnell, B. N. (1970). Septal ablation and the social behavior of the golden hamster. *Physiology and Behavior, 5*, 79-88.

Svare, B. B. & Leshner, A. I. (1973). Behavior correlates of intermale aggression and grouping in mice. *Journal of Comparative Physiological Psychology, 85*, 203-210.

Swiergel, A. H., Takahashi, L. K., & Kalin, N. H. (1993). Attenuation of stress-induced behavior by antagonism of corticotropin-releasing factor receptors in the central amygdala in the rat. *Brain Research, 623*, 229-234.

Szabo, S. & Glavin, G. B. (1990) Hans Selye and the concept of stress. Ulcer pathogenesis as a historical paradigm. In D. E. Hernandez & G. B. Glavin (Eds.), *Annals of New York Academy of Science*, Vol 597. Neurobiology of stress ulcers (pp. 14-16). New York: New York Academy of Science.

Tornatzky, W., & Miczek, K. A. (1993). Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiology and Behavior, 53*, 983-993.

Tornatzky, W., & Miczek, K. A. (1994). Behavioral and autonomic responses to intermittent social stress: differential protection by clonidine and metoprolol. *Psychopharmacology, 116*, 346-356.

Uno H., Tarara R., Else J. G., Suleman M. A., & Sapolsky, R. M. (1989). Hippocampal damage associated with prolonged and fatal stress in primates. *The Journal of Neuroscience, 9,* 1705-1711.

Van Bockstaele, E. J. , Colagg, E. E. & Valentino, R. J. (1998). Amygdaloid corticortopin-releasing factor targets locus coeruleus dendrites: Substrate for the coordination of emotional and cognitive limbs of the stress response. *Journal of Neuroendocrinology, 10*(10), 743-757.

Van de Kar, L. D., Peichowski, P. E., Rittenhouse, R. A., & Gray, T. S. (1991). Amygdaloid lesions: Differential effect on conditioned stress and immobilization-induced increases in corticosterone and renin secretion. *Neuroendocrinology, 54*, 89-95.

Vazdarjanova, A., Cahill, L., & McGaugh, J. L. (2001). Disrupting basolateral amygdala function impairs unconditioned freezing avoidance in rats. *European Journal of Neuroscience, 14*(4), 709-723.

Vazdarjanova, A., McGaugh, J. L. (1999). Basolateral amygdala is involved in modulationg consolidation of memory for classical fear conditioning (1999). *The Journal of Neuroscience, 19*(15), 6615-6622.

Walker, D. L. & Davis, M. (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *The Journal of Neuroscience, 17*(23), 9375-9383.

Wallace, K. & Rosen, J. (2001). Neurotoxic lesions of the lateral nucleus of the amygdala decrease conditioned fear but not unconditioned fear of a predator odor: Comparison with electrolytic lesions. *The Journal of Neuroscience, 21*, 3619-3627.

Watanabe, T., Morimoto, A., Sakata, Y., Tan, N., Morimoto, K., & Murakami, N. (1992). Running training attenuates the ACTH responses in rats to swimming and cage-switch stress. *Journal of Applied Physiology, 73*, 2452-2456.

Weisencrantz, L. (1956). Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *Journal of Comparative Physiological Psychology, 49*, 381-391.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (2000). The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical fear conditioning. *The Journal of Neuroscience, 20*(18), 7059-7066.

Yodyingyuad, U., de la, R.C., Abbott, D. H., Herbert, J., & Keverne, E. B. (1985). Relationship between dominance hierarchy, cerebrospinal fluid levels of amine transmitter metabolites (5-hydroxyindole acetic acid and homovanillic acid) and plasma cortisol in monkeys. *Neuroscience, 16,* 851-858.

APPENDIX A

Literature Review

Stress: Historical Perspective

Stress can be defined as the body's attempt to reestablish physiological balance or homeostasis (Gilbert, 1998). A *stressor* can be defined as anything that disrupts one's physiological balance, while a *stress response* may be defined as a body's attempt to reestablish this balance (Moberg, 1985; Sapolsky, 1992). The term *stress* had an established usage long before its development as a physiological concept (Pollock, 1988) with the origins of this concept going back as far as the ancient Greeks (Chrousos, Loriaux, & Gold, 1986).

The notion of balance has been a pervasive theme in physiology and has become a common thread in the work of investigators whose research has provided the background for the physiological concept of stress. This concept of balance, later termed homeostasis, was addressed as early as 450 B. C. by the philosopher Empedocles (Chrousos et al., 1986) and, by the late nineteenth century, was expanded by Claude Bernard in his description of the evolutionary development of physiological systems to buffer the internal environment from environmental fluctuations (Kopin, Eisenhofer, & Goldstein, 1986). By the twentieth century, stress physiology had emerged as a true discipline, largely growing from the works of Walter Cannon and Hans Selye (Mason, 1975; Sapolsky, 1992).

It was Cannon who coined the term *homeostasis* (Asterita, 1985) which refers to the state of a system whereby coordinated physiological responses serve to maintain a relatively stable internal environment (Kopin et al., 1986). In his investigation of the physiological basis of homeostasis, Cannon studied the reaction of the sympathoadrenomedullary system to threatening situations (real or perceived) and, in doing so, demonstrated the role of epinephrine in stress physiology (Asterita, 1985;

Sapolsky, 1992). Cannon was the first to describe such an emergency reaction, which has become known as the "fight or flight" response. This response involves the activation of the sympathetic portion of the autonomic nervous system and the adrenal medulla, which releases the catecholamines epinephrine and norepinephrine in the bloodstream (Asterita, 1985; Kopin et al., 1986). The release of these catecholamines intensifies sympathetic activity (Kopin et al., 1986; Moberg, 1985).

The pioneering work of Cannon was followed by the efforts of Hans Selye (Asterita, 1985). It was Selye who first to used the term *stress* as it is used today (Chrousos, et al., 1986). Selye introduced the concept of stress to the biomedical community (Szabo & Glavin, 1990) and characterized the stress syndrome known as the general adaptation syndrome (GAS). GAS is an integrated, nonspecific syndrome in which the adrenal gland plays an important role in the body's attempt to counteract stressful stimuli (Selye, 1975). In 1936, while conducting a series of experiments using injections of ovarian extracts and other noxious stimuli, Selye noted that these substances caused a number of consistent changes including adrenal cortex hypertrophy, thymolymphatic atrophy, and ulceration of the gastric mucosa (Selye, 1976a). From this and further experiments Selye proposed the GAS consisting of the alarm reaction, the stage of resistance, and the stage of exhaustion (Selye, 1976b).

The alarm reaction is the first stage of the GAS. This acute phase involves the initial sympathetic release of catecholamines. The second stage, resistance, is the stage of adaptation or adjustment which may last minutes, hours, days, weeks, etc. During this stage, the physiological responses are an attempt by the body to maintain homeostasis in the presence of stressors (Asterita, 1985). If the stressor predominates, the organism lapses into the third stage, exhaustion. In this stage the attempts to maintain homeostasis exhaust bodily resources and this may lead to diseases such as cancer and cardiovascular disease and eventually death (Gilbert, 1998).

Although Selye proposed a pathological outcome of the GAS, he did not think that stress was inherently destructive (Gilbert, 1998). In fact, in the short term it provides metabolic resources for immediate needs. The stress response is a catabolic process that is characterized by the inhibition of energy storage and the mobilization energy resources for immediate needs. Both of these responses increase the level of blood glucose which is delivered more rapidly to needed areas by an increase in cardiovascular and cardiopulmonary tone. The stress response also includes suppression of the immune system, the inflammatory response, pain perception, digestion, and the reproductive system. In sum, anabolic ("energy storage") and energy consuming processes that are counterproductive are inhibited, while catabolic ("energy mobilizing") processes are increased to meet the immediate energy demand involved in reestablishing homeostasis (Sapolsky, 1992). However, prolonged stress can have deleterious effects including hypertension, ulcers, decreased resistance to disease, loss of bone mass, and suppression of reproductive function. Prolonged stress can also lead to the development of stress-related disorders such as posttraumatic stress disorder, depression, and anxiety disorders (Herman, Prewitt, & Cullinan, 1996; Sapolsky, 1992).

Fear and Stress

Similar to stress, fear is an intervening variable that has been used to describe a collection of physiological and behavioral changes that occur when an organism is confronted by threatening events (Davis, 2000). Fear is a reaction to an actual threat and typically is a short-term response. The constellation of responses that take place when an individual experiences fear are similar to the responses that are seen when an individual is exposed to stressful stimuli. It would seem that both fear and stress not only are described by a range of physiological and behavioral responses to threatening stimuli, but that they are also described by the emotional, or subjective, experience that follows this stimuli (Davis, 1997, 2000). While fear-producing stimuli and stressors are often one in

the same, this dual nature does not always apply. For instance while predators would evoke fear and stress responses, a food shortage would likely only evoke a stress response.

Pavlovian fear conditioning has been used extensively to investigate the neurobiological substrates of fear. This procedure involves pairing an initially neutral stimulus (conditioned stimulus) such as a light or a tone with an aversive stimulus (unconditioned stimulus) such as a foot shock that produces behavioral and physiological responses that are thought to be indicative of a fear state. After such pairings, the previously neutral stimulus is able to evoke a conditioned response that is comprised of a series of physiological and behavioral responses that are similar to those seen in response to the original aversive stimulus (Davis 1997, 2000).

Adrenal Medullary Stress Response

Generally, the body is in a state of physiological balance. When this homeostatic balance is challenged, stress responses are produced (Moberg, 1985) and may include behavioral, neuroendocrine, and autonomic changes (Asterita, 1985). These responses enable the body to reestablish homeostasis. According to Asterita, the autonomic nervous system is one of the first physiological pathways activated. Signals from the environment or the central nervous system reach the limbic system where they may be integrated with other relevant information. The information continues toward the cortical areas where analytical interpretation of the stimulus occurs. After the information is emotionally and rationally integrated, it is sent back to the limbic system where emotional arousal will result if the interpretation of the stimulus indicates danger. These signals reach the hypothalamus, which controls the autonomic nervous system; if the "flight or fight" response ensues, sympathetic activation of the adrenal medulla will cause the release of epinephrine and norepinephrine (Asterita, 1985).

The adrenal gland is considered to dominate the endocrine stress response. In response to a stressor, sympathetic projections stimulate epinephrine release (and norepinephrine to a smaller extent) from the core, or medulla, of the adrenal gland. Other sympathetic projections go to essentially every other organ and stimulate the release of norepinephrine (Sapolsky, 1992). Concurrently, stimulation of neuroendocrine cells in the hypothalamus culminates in adrenocorticotropin (ACTH) secretion; the HPA-axis is activated, resulting in the release of glucocorticoids from the adrenal cortex. Threatening circumstances may warrant vigorous activity, and the adrenal responses that accompany them assist in the mobilization of the body's energy resources (Sapolsky, 1992). With respect to the stress response, the purpose of adrenal activation is to provide the organism the ability to anticipate, react, and adjust to threatening stimuli, whether they are real or perceived (Asterita, 1985; Kopin et al., 1986; Moberg, 1985).

Other Neuroendocrine Responses to Stress

Various hormones are categorized as stress indices. In hamsters and rats, ACTH, glucocorticoids, ß-endorphin, ß-lipotropin, as well as norepinephrine and epinephrine have increased in response to various stressors (Bunnell, Meyerhoff, & Kant, 1988; Huhman et al., 1990; McCarty & Kopin, 1979; Natelson et al., 1987; Ottenweller, Tapp, Burke, & Natelson, 1985). An increase in pituitary cyclic AMP, a cyclic nucleotide, may be considered a stress index for it has been shown to increase in response to stress (Bunnell et al., 1988). Cyclic AMP serves as a second messenger in the release of many neurotransmitters and hormones and is involved in the synthesis and release of anterior pituitary hormones (Greengard & Kebabian, 1974; Kant, Meyerhoff, Bunnell, & Lenox, 1982; Luciano, Vander, & Sherman,1983).

ACTH, ß-endorphin, and ß-lipotrophin are derived from proopiomelanocortin (POMC) which is a large 265 amino acid residue glycoprotein (Axelrod & Reisine, 1984). The main secretagogue for ACTH is corticotropin-releasing hormone (CRH).

CRH is synthesized primarily by the paraventricular nucleus (PVN) and the anterior periventricular nuclei of the hypothalamus, but it is also synthesized by other regions of the hypothalamus, including the supraoptic, dorsolateral, and ventromedial nuclei (Bennit & Whitehead, 1983; Brown, 1994). CRH is released into the median eminence and is carried via the hypothalamic portal system to the anterior pituitary where it stimulates the release of the POMC peptides. ACTH release is modulated by oxytocin, vasopressin, and catecholamines (Axelrod & Reisine, 1984; Makara, 1985). ACTH is carried through the bloodstream to cells of the zona fasiculata layer of the adrenal cortex where it stimulates the release of the glucocorticoids cortisol and/or corticosterone. Through negative feedback control, these glucocorticoids inhibit ACTH and CRH release (Brown, 1994).

Though increases in the plasma levels of certain hormones occur in response to a stressor, some hormones are more sensitive indices of stress than others (Natelson et al., 1981; Natelson et al., 1987). In investigating neuroendocrine responses to a graded stressor (shock), catecholamines were reported to be sensitive and reliable indices of stress; graded stress intensities produced corresponding, monotonic increases in plasma levels of epinephrine and norepinephrine. Corticosterone and cortisol were reported to reliable indices in that a graded increase in stressor intensity consistently produced a corresponding, nonmonotonic increase in these plasma levels. Though catecholamines appear to be both sensitive and reliable indices of stress, they are not always ideal. Catecholamines have a short half-life (1 to 3 min) and become less sensitive as the period between the experimental trial and blood collection increases (Natelson et al., 1981; Natelson et al., 1987). Considering neither catecholamines nor glucocorticoids can be ideal stress indices (Natelson, Holaday, Meyerhoff, & Stokes, 1975) multiple indices of stress may be warranted in studies assessing stress responses.

While vasoactive intestinal peptide, vasopressin, and thyrotropin releasing hormone are important prolactin-releasing factors (McCann, 1988; Reichlin, 1992),

inhibition of hypothalamic dopamine plays a primary role in prolactin secretion (Arahaf, Kailani, & Selman, 1992) for it inhibits prolactin synthesis and release (McCann, 1988). Opiates appear to regulate prolactin secretion through inhibition of dopamine (Arahaf et al., 1992), though it has been reported that ß-endorphin stimulates the transcription of prolactin mRNA (Black, 1992).

In general, neuroendocrine responses to various stressors have been well-investigated. For example, the levels of plasma catecholamines, prolactin, corticosterone, and ACTH of rats increase in response to various stressors such as shock, immobilization, novel environment exposure, and running (Deturk & Vogel, 1980; Natelson et al., 1987; Natelson et al., 1981, Seggie & Uhlir, 1979; Watanabe et al., 1992). Plasma levels of corticosterone and prolactin increase after 3-min novel environment exposure and, when compared to 5 sec of handling, it elicits a greater stress response—the corticosterone level is higher in response to novelty than in response to handling (Seggie & Uhlir, 1979; Seggie, Uhlir, & Brown, 1974). Interestingly, in rats prolactin levels have increased after stress exposure while in golden hamsters prolactin has either decreased (Huhman et al., 1995) or has remained unchanged (Huhman et al., 1990) in response to social defeat. Neuroendocrine and other responses to social stress will be discussed below.

Agonistic Behavior

Agonistic behaviors have been defined as a class of behaviors –aggressive, submissive, and defensive—that occur in competitive situations between conspecifics involving resources, mates, and space. The term *aggression* has been used to categorize a variety of behaviors including actions by which one individual either has caused or threatens to cause physical injury to another (Leshner, 1975). Submissive and defensive categories of behavior have been used to classify a range of behaviors like flight, and avoidance that are exhibited by defeated or subordinate animals in agonistic encounters.

Clear distinctions between submissive and defensive behaviors and defensive and aggressive behaviors have not always been achieved in the literature (Adams, 1979; Blanchard & Takahashi, 1988). Adams (1979) makes a distinction between submission and defense and has proposed separate but parallel neural pathways for these categories of behavior. A more unitary concept of defense and submission has also been proposed (Blanchard & Blanchard, 1979) and has been used in the quantification of agonistic behavior (Jasnow & Huhman, 2001; Jasnow et al., 1999).

Hormones and Agonistic Behavior. There are data to support the proposition that different hormones modulate different classes of agonistic behavior. High ACTH levels have been shown to decrease aggressiveness and increase fearfulness in the presence of a novel conspecific, while intermediate pituitary-adrenocortical hormone levels suggest that the animal is predisposed to be more aggressive and less fearful (Brain, 1972b, Leshner, 1975; Svare & Leshner, 1973). Using mice, Nock and Leshner (1976) found that inhibiting changes in pituitary-adrenocortical and pituitary-gonadal hormones delayed the decrease in aggressive behaviors and the increase in submissive behaviors that are generally associated with the establishment of a dominant-subordinate relationship. Preventing defeat-induced changes in corticosterone delayed changes in submissiveness, while preventing changes in testosterone had no effect on agonistic responses of defeated mice. From these findings, Nock and Leshner proposed that ACTH is the primary hormone that mediates the effects of defeat on aggressive response, whereas corticosterone is the primary hormone that mediates the effects of defeat on submissive behavior. Correspondingly, Leshner and Politch (1979) found that an increase in ACTH was followed by increased submissive behavior in intact versus adrenalectomized mice. The critical hormone involved in these changes appears to be corticosterone since the increase in submissive behavior was dependent upon the ability of the increase in ACTH to produce a subsequent increase in corticosterone. In sum, it

appears that hormonal responses to defeat may be a part of the physiological system that mediates the effects of defeat experience on agonistic behavior (Leshner, 1975).

How may hormones affect agonistic behavior? Some possible ways hormones may exert their effects on agonistic behavior by modifying central nervous system circuits that control interpretation of agonistic stimuli and integration of agonistic responding, the general physiological state of the organism, or peripheral sensory receptors (Leshner, 1975). Hormones most likely influence agonostic behavior through direct modification of the state of brain systems like the limbic system, which may involve mediation of the interpretation of the stimulus or stimuli and the integration of responses (Leshner, 1975). It has also been suggested that initial experiences in social situations may feedback through the endocrine system to modify the animal's perception and, in doing so, ongoing and subsequent behavior. Long-term changes in the animal's hormonal state as a result of the initial defeat experience may also modify future agonistic reactions (Leshner, 1975).

Interestingly, evidence exists which indicates that there can be a disassociation between behavioral and neuroendocrine responses to social stressors (Heinrichs et al., 1992, Pich et al., 1993). Alterations in the behavioral effects of social defeat have been investigated using the elevated plus maze. Following defeat by an aggressive resident, rats decrease the percent time spent in the exposed arms of the maze (Heinrichs et al., 1992; Pich et al., 1993). Similarly, Pich et al. found that peripheral application of an anti-CRF serum blocked the ACTH and corticosterone response in rats to social defeat stress. This change in HPA responsivity was not accompanied by changes in the stress-induced behavioral responses in an elevated plus maze.

In addition, intra-amygdaloid infusions of another CRF receptor antagonist α–helical CRF_{9-41} reversed the defeat stress-induced decrease in time spent in the open arms of the maze, but did not alter the corticosterone and ACTH response to defeat

(Heinrichs et al., 1992). Jasnow et al. (1999) found that peripheral administration of the CRH_1 receptor antagonist CP-154,526 significantly reduced the plasma level of ACTH in previously-defeated hamsters during a subsequent interaction with a non-aggressive intruder, but failed to alter the duration of submissive/defensive behavior produced in response to the non-aggressive intruder. The dissociation of these effects appears to contradict Leshner's (1975) proposal that hormone responses of defeat mediate the effects of defeat experience on ongoing agonistic behavior (Leshner, 1975).

Neuroanatomy of agonistic behavior. Offensive and defensive aggression appear to be regulated by different neural sites (Albert & Walsh, 1984; Blanchard & Blanchard, 1988). Data indicate that the amygdala, medial preoptic area (MPOA), anterior hypothalamus (AH), ventromedial hypothalamus (VMH), septum, periaqueductal gray (PAG), and bed nucleus of the stria terminalis (BNST) are neuroanatomical substrates of defensive aggression (Blanchard & Blanchard, 1988 for review). Lesions of the dorsomedial tegmentum and substantia nigra enhance offensive aggression (Albert & Walsh, 1984) while lesions of the ventromedial tegmentum abolish offensive aggression (Adams, 1987). Lesions of the septum and medial hypothalamus appear to indirectly decrease offensive aggression by increasing defensive aggression (Albert & Walsh, 1984). Delville, De Vries, & Ferris (2000) report that c-*Fos* immunoreactivity in the medial amygdaloid nucleus (MeA), VMH, BNST, and dorsolateral part of the midbrain central gray is increased in male hamsters who attack a conspecific intruders compared to hamsters who only experience the odor of conspecifics in their homecage. The AH has reciprocal connections with these areas, and the authors suggest that in hamsters the AH is an area of integration in a network of neural sites controlling offensive aggression.

Similar studies have provided information as to neural substrates of submissive behavior in the male Syrian hamster. Following acute defeat, c-*fos* mRNA expression in the medial nucleus of the amygdala is increased in both dominant and subordinate animals and

is increased in the supraoptic nucleus (SON) in dominant animals. In subordinate animals, c-*fos* expression is elevated in the cingulate cortex, lateral septum (LS), amydalahippocampal area (Ahi), septohypothalamic nucleus (Shy), dorsal periaqueductal gray (PAG), cuneiform nucleus (CnF), central nucleus of the amygdala (CE), anterior and intermediate subdivisions of the paraventricular nucleus of the hypothalamus (PVN), MPOA, AH, VMH, BNST, dorsal raphe, locus coeruleus, and arcuate nucleus of the hypothalamus (Kollack-Walker et al., 1997).

In addition, Kollack-Walker et al. (1999) found that chronic defeat decreases c-*fos* expression in the PVN (anterior subdivision), AH, CE, intermediate subdivision LS, SON, SHy, which the authors suggest is indicative of an habituation of physiological processes. No difference in c-*fos* expression was seen in AH, CnF, dorsal PVN, and lateral VMN in subjects experiencing chronic vs. acute defeat. These findings led the authors to propose that in male hamsters the stress of acute defeat activates distinct brain regions and that chronic defeat leads to a selective pattern of habituation of immediate early gene expression within certain brains regions. Changes in c-*fos* activation may reflect changes in neurotransmission within the HPA axis. In brain regions where no adaptation of c-*f*os expression was observed, this lack of change could be indicative of processes that are not as likely to adapt to repeated defeat such as defensive behavior (Kollack-Walker et al., 1999).

*Syrian hamsters, glucocorticoids, and agonistic behavior***.** Hamsters have been used in a wide variety of studies and are a good species to use in the study of stress responses (Huhman et al., 1990; Sodetz & Bunnell, 1970). The endocrine stress response of Syrian hamsters has been investigated (Huhman et al., 1990; Huhman et al., 1991; Huhman et al., 1992), and their agonistic behaviors and behavior patterns have been categorized (Grant & Mackintosh, 1963). Hamsters are ideal subjects in the social conflict paradigm for they readily initiate social activity with conspecifics, their behavior is easy to quantify, and their size enables sufficient blood samples for a number of

hormone assays (Huhman et al., 1990; Huhman et al., 1991; Schindler & Knigge, 1959). Interestingly, the agonistic behavior of Syrian golden hamsters is different from that of rats and mice. Compared to these other rodents, hamsters have been described as largely nonsocial. Rather than establishing dominance relationships that facilitate habitation in social groups (as seen with rats), the intraspecific fighting of hamsters appears to serve to chase other members of the same species away, and, therefore, to spread the population (Hanney, 1975*)*.

While both cortisol and corticosterone have been found to be reliable indices of stress, cortisol is more responsive than corticosterone in Syrian hamsters (Ottenweller et al., 1985). In hamsters, glucocorticoid levels reach a peak near the onset of the dark cycle (Albers et al., 1985); Frenkel, Cook, Grady, and Pendleton (1965) have reported that the ratio of cortisol to corticosterone in hamsters appears to increase in response to stress. When hamsters have been subjected to chronic stress, the cortisol level has elevated and the corticosterone-cortisol ratio has declined. In studying the effects of social conflict on glucocorticoids, Huhman et al. (1990) found that cortisol and corticosterone responses of male hamsters to defeat were comparable in magnitude. From these findings and in order to conserve plasma for other radioimmunoassays, limiting glucocorticoid assessment to that of cortisol may be justified when studies employ Syrian hamsters as test subjects.

Social Stress

There are two basic models of social conflict using rodents, the colony model and the resident-intruder paradigm. The colony model involves creating a semi-natural environment in which the co-habitation of males and females allows a social structure to develop over time (Blanchard et al., 1998; Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993). Typically, one of the males is recognized as dominant with the remaining males regarded as subordinate. Animals in this state of subordination

experience chronic social stress (Blanchard et al., 1998; Blanchard et al., 1993). Though living in social groups can be advantageous due to increased defense against predators, there is increased competition for limited resources such as food and mates (Martinez et al., 1998).

The resident-intruder paradigm is another common model of social stress and consists of placing a male in the home-cage of another male. In this model, territorial aggression is the basis for social conflict and, typically, the resident defeats the intruder. Various methods and manipulations have been used to ensure that defeat of the intruder will occur (Martinez, Calvo-Torrent, & Pico-Alfonso, 1998 for review). For hamsters, a resident-intruder encounter leads to the rapid establishment of a dominance relationship, usually within 2 min of the initial confrontation. The defeated animals show a variety of submissive postures and behaviors that may ward off further attack (Grant & Mackintosh, 1963) and increased pituitary-adrenocortical activity and lowered gonadal activity; both changes are indicative of stress (Huhman et al., 1990, 1991).

Physiological responses to social stress. Not all categories of agonistic behavior have been associated with a neuroendocrine stress response. Social conflict has been shown to increase HPA activity in defeated and subordinate animals. *P*lasma ACTH (Huhman et al., 1990, 1991) and corticosterone levels increase after acute defeat, repeated defeat, or subordination (Blanchard et al., 1993; Pich, 1993, Huhman et al., 1990, 1991). Specifically, Huhman et al. (1991) found that submissive, but not dominant, male golden hamsters exhibited potentiated plasma levels of ß-endorphin, ACTH, and cortisol following 1 and 5 exposures to a dominant male conspecific. Importantly, fighting, itself, is not considered a biologically relevant stressor because "winning" has not been associated with a neuroendocrine stress response (Huhman et al., 1990). Though data indicate that dominant hamsters do not exhibit potentiated neuroendocrine activity following a social encounter, Kollack-Walker et al. (1998)

postulate there may be an initial increase in CRH mRNA in dominant hamsters that quickly returns to baseline. In sum, it appears that specific behavior patterns (i. e., submissive/subordinate behavior) are associated with a neuroendocrine stress response and, hence, defeat can be considered to be a significant stressor.

Changes in HPA activity are only one of the endocrine responses to social stress. An increase in adrenomedullary activity is also associated with social stress. For instance, an increase in norepinephrine and epinephrine has been seen following acute defeat (Brain, 1980; Sgoifo, de Boer, Huller, & Koolhaas, 1996). Defeat and subordination also decrease activity of the hypothalamic-gonadal-axis (Blanchard et al., 1993; Huhman et al., 1991). This alteration in HGA activity following defeat has been demonstrated by a decrease in lutenizing hormone, follicle-stimulating hormone, and testosterone (Bronson, 1973; Blanchard et al., 1993; Huhman et al., 1991; Schuurman, 1980).

Autonomic and physical changes are also associated with social stress. Defeat and subordination have produced increases in heart rate and blood pressure (Bohus et al., 1990; Tornatzky & Miczek, 1993, 1994), and changes in body temperature (Tornatzky & Miczek, 1993, 1994; Meerlo et al., 1996a). Subordination typically leads to a decrease in body weight (Albonetti & Farabollini, 1994; Merlo et al., 1996b). In addition, the adrenal gland and the spleen are enlarged and the thymus is reduced in both dominant and subordinate rats, though the change is more marked in the subordinates (Blanchard et al., 1993; Blanchard et al., 1998).

The brain also changes as a result of social stress. Chronic stress can have damaging effects on the hippocampus including cell death and inhibition of neurogenesis. Chronic stress generates neurodegenerative effects in the hippocampus which appear to be due to prolonged glucocorticoid exposure (Sapolsky, Uno, Rebert, & Finch, 1990; Uno, Else, & Sapolsky, 1989). Neurogenesis in the dentate gyri has also been suppressed

by acute and repeated stress. These effects on the hippocampal formation have been associated with impairments in hippocampal-dependent learning (Gould, Tanapat, Rydel, Hastings, 2000). Social stress also effects the dopaminergic, noradrenergic, serotonergic glutamatergic and GABA neurotransmitter systems. The effects on dopamine include changes in dopamine levels, metabolism, and turnover (see Martinez et al., 1998 for review). Social stress also increases adrenal noradrenergic activity by increasing tyrosine hydroxylase (TH) mRNA and TH levels in the locus coeruleus (Wantanabe et al., 1995), hippocampus, and medulla (Serova, Bozlova, Rivkin, & Naumenko, 1992). It seems that in general, stress exposure increases the concentration of the neurotransmitter serotonin (5-HT) and its metabolite 5-hydroxyindole acetic acid (Yodyingyuad et al., 1995; Blanchard et al., 1993; Berton et al., 1998; Delville, Melloni, & Ferris, 1998; Amat et al., 1998a, 1998b; Maswood et al., 1998; Grahn et al., 1999). In addition, social stress is associated with an increase in 5-HT_{2A} receptor binding (Flugge, 1995) and a decrease in 5-HT_{1A} receptor binding (McKittrick et al., 1995). The amino acids glutamate and GABA are also affected by social stress in that it increases $GABA_A$ receptor subunits in mRNAs (Kang et al., 1991) and increases the ratio of NMDA/AMPA in the hippocampus (Kruger et al., 1993).

Stress can also suppress immune system function. For example, rats and hamsters that have experienced social defeat show a decrease in serum antibodies (Fleshner et al., 1989; Jasnow et al., 2001). In addition, repeated and continuous defeat decreases T-cell proliferation (Hardy, Quay, Livnat, & Ader, 1990) and thymus weight (Blanchard et al., 1993). Stress can create serious health challenges with respect to suppression of immune function for prolonged stress is associated with impaired resistance to disease (Sapolsky, 1992).

Psychological stress and conditioned defeat. Elicitation of stress responses are not limited to physical stressors such as shock. The body responds to both physical and

psychological stressors. Returning an animal to a situation in which it was previously exposed to aversive stimulation elicits a pattern of neuroendocrine stress responses that is similar to patterns seen following exposure to the actual stressor, even though the stressor is not present (Huhman et al., 1992). Psychological stress has been documented in both rodents (Huhman et al., 1992) and man (Meyerhoff, Oleshansky, & Mougey, 1988). In hamsters, psychological stressors such as the presence (no contact) of a dominant conspecific have been reported to increase the levels of prolactin, cyclic AMP, ß-endorphin, and cortisol (Bunnell et al., 1988; Huhman et al., 1992).

These studies indicate that there is an important psychological component to the HPA activation in hamsters who have experienced defeat (Huhman et al., 1992). Other studies have indicated that defeat experiences may affect avoidance learning (Hudgens & MacNeil, 1970) and alter responses during future social experiences (Hebert et al., 1996; Potegal et al., 1993). Hudgens and MacNeil (1970) found submissive mice learned a two-way avoidance task significantly faster than dominant mice. Potegal et al. (1993) report a behavioral change in previously-defeated male golden hamsters. Subsequent to defeat by a larger and more aggressive hamster, normal aggressive behavior of a resident hamster toward a non-aggressive intruder is replaced by defensive-submissive behaviors. This behavioral change has been called "conditioned defeat" (Potegal et al., 1993). Similar responses have been seen in rats. Seward (1945) found that when defeated rats were re-exposed to a winning opponent, the frequency of aggressive behaviors and advances to the opponent decreased. This change in behavior was also observed during agonistic encounters with a previously-submissive opponent.

Amygdala

The term "limbic system" arose from the concept of the limbic lobe presented by Broca in 1878. Broca introduced the term "limbic lobe" to designate brain tissue that surrounds the brainstem and lies below the neocortical mantle (Isaacson, 1982a). Papez

developed a hypothesis for a neural circuit for emotions which was found to be reminiscent of Broca's "limbic lobe". This circuit included the mammillary body, thalamus, cingulated gyrus, and hippocampus. Other regions, including the amygdala, have been found to have a modulatory role in emotional behavior. Papez's theory stimulated further research in the neural regulation of emotions, but this research has suggested that no "system" truly exists—the boundaries are fuzzy. What can be included in this "system" can vary depending upon the behavior under study, and it overlaps with many other systems in the brain (Isaacson, 1993, Shepard, 1994). But, at a general level, the limbic system concept is useful in generating hypotheses and describing behavior in relation to the interaction of more-or-less specific anatomical areas (Isaacson, 1982b, 1993). Though the boundaries may be "fuzzy" and, thereby, the structures listed as being limbic vary, it is generally conceded that the septal area, hippocampus, amygdala, and cingulate cortex are part of the limbic system.

In primates, the amygdala is an elliptical mass of gray matter located in the medial part of the temporal lobe. It borders the rostral end of the hippocampus and the anterior end of the lateral ventricle (Alheid, de Olmos, & Beltramina, 1995). Multiple themes have been used to categorize the different regions/nuclei of the amygdala. Traditionally, the amygdala has been divided in the basolateral and corticomedial divisions (Isaacson, 1982a). Based on histochemistry and connectivity, the amygdala can be divided into an olfactory amygdaloid group, a basolateral amygdaloid group, a medial amygdaloid group, and a central amygdaloid group (De Olmos et al., 1985).

Another categorization includes the concept of the "extended" amygdala in which the amygdala is expanded to include the bed nucleus of the stria terminalis (BNST). The BNST, medial nucleus of the amygdala, and the central nucleus of the amygdala (CE) have similar fiber connections and many identical immunoreactivities (De Olmos et al., 1985). The medial portion of BNST and the lateral portion of the BNST form anatomical

and possibly functional entities with the medial nucleus and CE nuclei, respectively (Dunn, 1987).

The amygdala and the autonomic nervous system. In humans, amygdalectomy leads to decreased skin conductance, hand temperature, and frontal electromyography, all of which are consistent with a fall in sympathetic activation (Lee et al., 1988). Stimulation of the amygdala has lead to autonomic reactions of that are indicative of fear, such as changes in respiration; lesions of the amygdala have produced deficits in fear conditioning as measured by galvanic skin response (see Davis, 1997 for review). CE lesions have blocked conditioned changes in heart rate and blood pressure (Davis, 1997).

Gastric ulcers may occur in response to stress and can be influenced by the amygdala. In the of study gastric pathology in response to immobilization, Henke (1980a) reported that inhibitory and facilitative systems exist within the amygdala. Bilateral lesions of the posterolateral amygdala significantly increased restraint-induced gastric pathology; gastric ulcers and hemorrhaging increased in severity and number. Dorsomedial lesions were associated with a reduction in gastric pathology, and combined lesions produced less gastric pathology than observed in controls. The results led Henke (1980a) to propose that the posterolateral areas of the amygdala are inhibitory and the dorsomedial areas of the amygdala are excitatory with respect to gastric pathology associated with physical restraint stress.

Henke (1980b) also discovered that the stimulation of the centromedial amygdala was associated with severe gastric pathology. Lesions to the ventral amygdalofugal pathway prevented pathology previously found by stimulation of the medial amygdala; lesions to the stria terminalis did not. The stria terminalis and ventral amygdalofugal pathways are major amygdaloid projections that connect the amygdala with other diencephalic, telencephalic, and lower brainstem regions. Henke's findings indicate that the medial amygdala, chiefly the central nucleus, initiates gastric pathology, and that

these effects may be transmitted by the ventral amygdalofugal pathway (Henke, 1980b**,** 1982). In 1992, Henke concluded that a temporal lobe neural loop (involving the hippocampus, entorhinal cortex, and amygdala as the nodal point) may influence the degree to which threatening experiences produce gastric pathology.

The amygdala and intraspecies/interspecies behavior. Klüver and Bucy (1937) and Weisencrantz (1956) provided early evidence necessary to indicate the amygdala is involved in emotional behavior. Following temporal lobe lesions, Klüver and Bucy found that subjects exhibited a constellation of behavioral changes that included hyperorality, hypersexuality, faulty visual recognition, and emotional blunting. Through the efforts of Weisencrantz, it was determined that the amygdala was they key area producing the emotional changes.

Manipulation of the amygdala has increased, decreased, and had no effect on agonistic behavior, which may be explained by differences in experimental procedures, context, dependent measures, and amygdala sub-nuclei that are involved (Blanchard & Takahashi, 1988; Bush & Barfield, 1974; Seigal & Edinger, 1983*).* Data suggest that the amygdala is not a neuroanatomical substrate for offensive aggression in the rat. In a resident-intruder test, Oakes and Coover (1996) did not find a change in offensive behaviors following medial, basolateral, and CE lesions, but rats generally showed very little aggression. Similarly, bilateral lesions of the amygdala (multiple nuclei) in resident male rats did not alter aggressive behavior when they were confronted by a male conspecific. In general, there were no lesion effects on agonistic behavior (Bush & Barfield, 1974). Similar results were found by Blanchard and Takahashi (1988); amygdala lesions had no effect on intermale aggression, but freezing behavior was reduced.

The stimulation of the rostral, lateral, and the central nuclei of the amygdala has been associated with the production of fear (Kling & Brothers, 1992 for review). What

has been described as a decrease in fear has been associated with lesions of the amygdala. In rats, lesions significantly attenuate the duration of freezing in response to a predator (Blanchard & Takahashi, 1988). A significant decrease in the frequency of evasive behaviors (movements of the forepart of the body or head and retreat to move away from another animal) has been seen following amygdala lesions (Kolb & Nonneman, 1973). In wild rats, lesions of the basolateral and corticomedial nuclei have decreased two forms of flight from a human intruder, flight distance and pursuit latency. From these results it is proposed that flight behavior is diffusely represented within the amygdala nuclei, and it is speculated that flight behavior of wild rats may be significantly reduced following damage to all major areas of the amygdala (Kemble et al., 1990).

The medial amygdaloid nucleus appears to be a major amygdaloid area involved in the modulation of defensive aggression in the rat. Defensive attack behaviors (vocal, defensive upright, jump, attack, and bite) were significantly decreased following lesions of the centromedial nucleus of the amygdala (Kemble et al., 1990). Lesions restricted to the medial nucleus decreased defensive biting without affecting flight behavior (Kemble, Blanchard, Blanchard, & Takahashi, 1984). In rats, medial, but not basolateral nor central, amygdaloid lesions decreased muricide and emotional responsiveness (including aggressive and defensive-like behaviors) induced by isolation, delta-9-tetrahydrocannabinol and olfactory bulbectomy (Shibata, Yamamoto, & Ueki, 1982).

Kolb and Nonneman (1974) reported that amygdala lesions in rats abolished shock-induced aggression and decreased contact time in a large arena. The behavioral category *aggression* was comprised of both aggressive and defensive behaviors and body positions. According to Blanchard and Takahashi (1988), behaviors that have been altered following amygdala lesions tend to reflect a change in defense rather than offense. It has been speculated that both basolateral and rostral CE may modulate defense

behaviors for lesions of these two areas were associated with decreased shock-induced aggression (Oakes & Coover, 1996).

Though not as well-studied as in the rat, the role of the amygdala in the agonistic behavior of the Syrian hamster has been investigated. In male hamsters, lesions of lateral amygdala nuclei appear to suppress shock-induced fighting (Shipley & Kolb, 1977). Bunnell et al. (1970) found that hamsters with large amygdaloid lesions exhibited a decrease in the amount of social contact with conspecifics. Following these lesions, subjects with defeat experience were less submissive and subjects with "winning" experience were less dominant. In addition, lesioned subjects that were socially inexperienced showed less aggressive or submissive behavior compared to preoperatively aggressive or submissive lesioned animals, respectively (Bunnell et al., 1970). Current findings concerning the CE and agonistic behavior will be discussed below.

The amygdala and neuroendocrine modulation. Numerous studies have demonstrated the importance of the amygdala in the mediation of the neuroendocrine stress response (Herman, Prewitt, & Cullinan, 1996). Indicative of this modulatory role, stimulation and lesioning of amygdaloid nuclei have been followed by alterations in neuroendocrine activity (Dunn, 1987; Dunn & Whitner, 1986; Matheson, Branch, & Taylor, 1971; Feldman, Conforti, & Saphier 1990; Prewitt & Herman, 1994; Seggie, 1983). The amygdala may modulate neuroendocrine stress responses via direct or indirect projections to the PVN (Gray et al., 1989). An anterograde tracing study has indicated that the medial CE projects to the medial and lateral parvocellular regions of the PVN which contain CRF, vasopressin, and oxytocin neurons, all of which are known to modulate the release of ACTH from the anterior pituitary (Gray et al., 1989). The majority of the amygdaloid projections to the PVN may actually be indirect. Because more recent tracing studies have indicated possible interactions between cortical, medial, central, and posterior amygdaloid nuclei and the BNST, medial preoptic area, and cells in

the anterior hypothalamus that project the PVN (Prewitt & Herman, 1994). Correspondingly, stimulation of the medial nucleus of the amygdala has increased plasma cortisol, and this increase may be blocked by bilateral lesions of medial preoptic nucleus, the stria terminalis, or the BNST (Feldman et al., 1990).

Various studies have indicated that electrical stimulation of the amygdala increases adrenocortical response while amygdala lesions inhibit this response (see Feldman et al., 1990 for review). Other studies have shown that subdivisions within the amygdala are differentially involved in adrenocortical functions (Herman et al., 1996). Matheson et al. (1971) found that stimulation of the corticomedial, basal, and lateral amygdaloid nuclei of cats facilitated the release of cortisol and corticosterone and that, in a few cases, corticomedial stimulation inhibited the glucocorticoid response. Using rats, Dunn and Whitner (1986) demonstrated that stimulation of CE and lateral nucleus of the amygdala decreased plasma cortisol. Though these findings do not agree with those from the Matheson et al. (1971) study, Dunn and Whitner used anesthetized rats which may interfere with normal HPA activity and reactivity.

Lesions studies have also provided information concerning the role of the amygdala in neuroendocrine activity. Resting hormone levels of corticosterone, growth hormone, and prolactin are reported to be unaffected by such lesions (Beaulieu et al., 1986; Seggie, 1979; Seggie, 1980). Seggie and others have investigated the effects of amygdala lesions on neuroendocrine stress levels. In 1979, Seggie found that basolateral lesions of the amygdala potentiated the corticosterone response in rats following exposure to 3 min novel environment stress and 5 sec of handling. Seggie (1983) reported that rats with corticomedial amygdala lesions were hyperreactive, but neither the corticosterone nor prolactin levels following exposure in response to environmental and handling stress were significantly different from that of controls. While lesions of the medial and central amygdaloid nuclei have led to blunted ACTH and corticosterone

responses to photic and acoustic stimuli, lateral amygdala lesions have not been shown to alter HPA activity in response to sensory stimulation (Feldman, Conforti, Itzack, & Weidenfeld, 1994). Effects of CE lesions on neuroendocrine responses will be discussed in the next section.

Central nucleus of the amygdala

The CE is part of the central amygdaloid nucleus group (De Olmos et al., 1985). It has been implicated in neuroendocrine (Roozendaal et al., 1991a,b; Van de Kar et al., 1991), and behavioral stress responses (Goldstein et al., 1996; Lee & Davis, 1997; Roozendaal et al., 1991b; Swiergiel et al., 1993). These responses have been both acute (unconditioned) and conditioned.

Unconditioned and conditioned behaviors. Studies have provided information as to the effect of CE manipulation on unconditioned and conditioned behavior. Unconditioned behavioral stress responses such as post-shock immobilization (Kim, Rison, & Fanselow, 1993; Roozendaal et al., 1991a) and avoidance of an anesthetized predator (Blanchard & Blanchard, 1972) have been attenuated following CE lesions. In contrast, Goldstein et al. (1996) and Holahan & White (2002) did not find post-shock decrements in freezing associated with CE lesions.

Various conditioned behaviors have also been altered following CE manipulation. Holahan and White (2002) reported that conditioned modulation and conditioned avoidance were impaired following electrolytic lesions of the CE. Electrolytic (Oakes & Coover, 1997) and ibotenic acid (Jellestad et al., 1986) lesions of the CE have produced deficits in passive avoidance behavior. In addition, Roozendaal, Bohus, and Koolhaas (1990) found that following social defeat, rats with post-training lesions of CE showed conditioned immobilization responses toward a dominant rat. These responses were similar to those seen in subjects serving as controls.

Jasnow, Davis, and Huhman (in preparation) reported that unilateral electrolytic lesions of the CE decreased submissive/defensive behavior in previously-defeated hamsters during conditioned defeat testing. In addition, subjects with both unilateral CE lesions and a CRH receptor antagonist infused into the bed nucleus of the stria terminalis (BNST) contralateral to the lesion site exhibited significantly less submissive/defensive behavior compared to lesion/drug controls. These results suggest that CRH acts within the BNST to modulate agonistic responses to social defeat and that the CRH originates within the neurons of the CE (Jasnow et al.). In addition, Jasnow and Huhman (2001) have shown that pre-training infusions of muscimol into the CE decreases submissive behavior during an encounter with a non-aggressive intruder. In animals that were previously defeated, the pre-testing infusion of muscimol decreased submissive behavior towards a non-aggressive intruder.

Fear Conditioning. As previously discussed, fear is a complex set of behavioral and physiological reactions to threatening stimuli. In the laboratory, fear conditioning has typically involved the pairing of an unconditioned, aversive stimulus such as a foot shock with a neutral stimulus such as a light or tone. After multiple pairings the previously neutral stimulus now elicits a host of behavioral and physiological responses that typically occur when the unconditioned stimulus is presented alone (LeDoux, 2000).

The fear-potentiated startle paradigm measures conditioned fear by an increase in the amplitude of the startle reflex in the presence of conditioned stimuli previously paired with shock (Davis, 1998). Previous research indicates that the central, lateral, and basolateral nuclei of the amygdala are important in acquisition and expression of fear-potentiated startle. Lesions of the central nucleus block the expression of fear-potentiated startle when either an auditory (Hitchcock & Davis, 1986) or visual conditioned stimulus (Campeau & Davis, 1995) is used. In addition, infusion of non-NMDA glutamate receptor antagonists into the central nucleus blocks the expression of fear-potentiated

startle to a visual or auditory cue (Kim, Campeau, Falls, & Davis; 1993; Walker & Davis, 1997). Pre- and post-training NMDA lesions of the lateral and basolateral nuclei block fear-potentiated startle (Sananes & Davis, 1992) to a visual conditioned stimulus. Lesions of these types also block fear-potentiated startle using an auditory conditioned stimulus (Campeau & Davis, 1995).

Other studies have also used conditioned responses to auditory/contextual cues previously paired with foot shock to assess involvement of various amygdala nuclei in conditioning to aversive stimuli. Based on extensive evidence, it is clear that the amygdala is plays an important role in the conditioned fear responses (Davis, 2000; LeDoux, 2000). Large amygdala lesions that include the CE produce decrements in contextual/auditory conditioning. Subjects with large RF lesions of the amygdala (including the CE) show attenuated freezing responses to a context previously paired with shock (Blanchard & Blanchard, 1972). Helmstetter (1992) found that post-training lesions of the amygdala (including the central, lateral, and basolateral nuclei) significantly reduce immobilitization to a conditioned contextual cue (shock-box). Goldstein et al. (1996) reported that pre-training and post-training NMDA amygdala lesions (including CE and BLA) block conditioned freezing to tone-context conditioned stimuli.

Localized CE lesions also produced deficits in conditioned freezing. Pre-training electrolytic (Nader et al., 2001) and NMDA (Goosens & Maren, 2001) lesions prevent freezing behavior in an auditory fear conditioning task. Goosens and Maren (2001) also demonstrated that these neurotoxin lesions attenuate freezing to contextual stimuli. Pre-training (Holahan & White, 2002) and post-training (Kim & Davis, 1993) electrolytic lesions of the CE impair freezing to a tone CS (Holahan & White, 2002; Kim & Davis, 1993); pre-training lesions also impair freezing to a tone-context CS (Holahan & White, 2002).

The basolateral amygdaloid complex (BLA) is also involved unconditioned and conditioned fear. Data indicate that the amygdala , and specifically the BLA, could be necessary for performance of unconditioned responses to aversive stimuli (Vazdarjanova, et al., 2001; Wallace & Rosen, 2001), as well as play a role in acquisition and maintenance of specific CS-US associations (Antoniadis & McDonald, 2001; Maren, 1999; Goosens & Maren, 2001; Vazdarjanova & McGaugh, 1999; Wilensky et al.,, 2000) and the expression of conditioned fear (Cousens & Otto, 1998). In addition, it has been proposed that the BLA is a site of storage or plasticity of such memories (Fanselow & LeDoux, 1999; Maren, 1999, 2001), though some authors disagree (Cahill et al., 1999; Fanselow & LeDoux, 1999).

Neuroendocrine modulation. With respect to neuroendocrine activity, CE lesions attenuate (Goldstein, Rasmusson, Bunney, & Roth, 1996*;* Hebert et al., 1993; Marcilhac & Siaud, 1996; Prewitt & Herman, 1994; Roozendaal, et al., 1992; Roozendaal et al., 1991a; Van de Kar et al., 1991) or leave unmodified (Hebert et al., 1993; Marcilhac & Siaud, 1996; Roozendaal, et al., 1992) various neuroendocrine responses to unconditioned and conditioned aversive stimuli. Electrolytic lesions of the CE attenuate neuroendocrine responses (epinephrine, norepinephrine, corticosterone, and prolactin) to a single foot shock (Roozendaal et al., 1991a). Axon-sparing ibotenic lesions of the CE inhibit the increase in plasma corticosterone after exposure to both conditioned foot shock stress and unconditioned immobilization (Van de Kar et al., 1991), as well as attenuate the plasma ACTH response to unconditioned immobilization (Prewit & Herman, 1994). In addition, N-methyl-D-aspartate (NMDA) lesions of the CE block the corticosterone response to contextual cues that have been previously paired with foot shock (Goldstein et al., 1996).

Interestingly, the area of CE damage, the timing of lesion, and the sparing of other amygdala nuclei may be important in CE lesion effects on neuroendocrine responses.

Marcilhac and Siaud (1996) found that lesions restricted to the medial division of the CE attenuate ACTH response to restraint stress, and lesions restricted to the lateral division of the nucleus have no effect. Roozendaal et al. (1992) found that pre-training but not post-training lesions of the CE (that included BLA damage) prevent an increase in corticosterone and prolactin to exposure to a context previously paired with shock. Conversely, Hebert et al. (1993) reported that radio-frequency lesions (RF) of the CE of rats did not alter neuroendocrine responses to acute or repeated foot shock stress, though CE lesions did attenuate increases in plasma levels of ACTH and prolactin in response contextual cues associated with foot shock. Basal hormone levels (Marcilhac & Siaud, 1996; Roozendaal et al., 1991a; Prewitt & Herman, 1994) are unchanged following CE lesions.

APPENDIX B

Experiment 1: Mean Duration of Agonistic Behavior (sec)

	Amygdala Lesion							
	Large		Small		Unilateral		Control	
Behavior	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*	*M*	*SEM*
Submissive/Defensive	305.94	38.69	444.32	47.38	370.98	58.03	439.81	35.00
Aggressive	2.11	4.24	1.52	5.19	31.18	6.35	2.26	3.83
Social	137.14	24.91	75.45	30.50	144.20	37.76	99.74	22.53
Nonsocial	154.80	37.33	78.38	31.52	53.65	14.12	48.80	10.36

APPENDIX C

Experiment 1: Mean Plasma Levels of ACTH (pg/ml) and Cortisol (μg/dl) in Response to a Resident Aggressor

	Amygdala Lesion			
	Lesion		Control	
	M	*SEM*	*M*	*SEM*
ACTH				
Acute Defeat	455.48	32.79	548.79	40.76
Baseline	110.89	30.23	163.09	39.03
Cortisol				
Acute Defeat	7.16	0.50	8.40	0.66
Baseline	2.04	0.49	2.39	0.64

APPENDIX D

Experiment 2: Mean Duration (sec) of Agonistic Behavior Towards a Resident Aggressor

	Amygdala Lesion			
	Lesion		Control	
Behavior	*M*	*SEM*	*M*	*SEM*
Submissive/Defensive	351.92	34.09	395.24	52.15
Aggressive	2.44	1.23	0.00	0.00
Social	146.81	22.49	115.29	29.77
Nonsocial	98.81	22.75	89.47	25.87
Opponent Aggression	184.60	29.50	173.50	30.11

APPENDIX E

Experiment 2: Mean Duration (sec) of Agonistic Behavior in Response to a Non-aggressive Intruder

	Amygdala Lesion			
	Lesion		Control	
	M	*SEM*	*M*	*SEM*
Submissive/Defensive				
Conditioned Defeat	20.38	3.72	92.44	11.96
No Defeat	8.74	4.62	38.31	23.60
Aggressive				
Conditioned Defeat	9.24	5.19	1.03	1.03
No Defeat	26.64	8.06	45.19	24.44
Social				
Conditioned Defeat	270.60	24.79	258.09	23.31
No Defeat	196.34	25.82	321.01	29.97
Nonsocial				
Conditioned Defeat	299.77	27.69	248.30	16.65
No Defeat	367.64	32.48	195.70	35.93
Locomotor/Exploratory				
Conditioned Defeat	242.89	25.82	152.10	23.82
No Defeat	269.88	26.73	80.73	29.59

APPENDIX F

Experiment 2: Mean Plasma Levels of ACTH (pg/ml) and Cortisol (μg/dl) in Response to a Non-aggressive Intruder

	Amygdala Lesion			
	Lesion		Control	
	M	*SEM*	*M*	*SEM*
ACTH				
Conditioned Defeat	177.99	23.67	242.40	37.43
No Defeat	189.74	24.50	227.39	34.65
Cortisol				
Conditioned Defeat	3.71	0.61	4.96	0.63
No Defeat	4.00	0.59	3.67	0.91