CONSEQUENCES OF MATERNAL ABUSE ON HPA AXIS FUNCTION, GROWTH, AND SOCIAL AND STRESS-INDUCED BEHAVIOR IN JUVENILE RHESUS MACAQUES

by

ALISON P. GRAND

(Under the Direction of Irwin Bernstein and Mar Sanchez)

ABSTRACT

Common outcomes associated with childhood maltreatment are aggression, anxiety, depression, alterations in the HPA stress response system, and growth alterations. This study utilized an animal model to examine the long term behavioral and physiological effects of maternal maltreatment. Subjects were 10 rhesus macaques *(Macaca mulatta)* that were maternally abused during the first 3 months of life and 10 non-maternally abused subjects. HPA axis activity was examined at 24, 30, and 36 months of age. Blood draws were collected at morning, noon, and night to investigate basal levels and the diurnal rhythm of cortisol. The dexamethasone suppression test was administered to examine negative feedback of cortisol, and the ACTH and CRF challenges tests were used to determine pituitary and adrenal sensitivity. HPA stress responses and behavioral responses to stressors were investigated through the use of 3 separate laboratory paradigms (neutral object test, fear-evoking object test, and human intruder paradigm) that exposed the subjects to stimuli of varying threatening intensities. To investigate growth alterations, physical growth measurements and blood samples for growth hormone analyses were collected when subjects were 36, 42, and 48 months of age. Subjects were also observed in their social groups to investigate aggression and affiliative behavior. The results

demonstrated that abused and control subjects differed in their behavioral responses to novel stimuli. Abused subjects explored and inspected novel objects more, inspected objects more quickly, avoided objects less, and were more likely to bite objects than control subjects. When subjects were exposed to a novel human making direct eye contact (highly threatening), abused subjects displayed more nervous behavior than controls. In the social group, abused subjects were more aggressive, but did not differ from controls with regard to the proportion of time spent in proximity or contact with group members. Contrary to hypotheses, abused subjects did not demonstrate HPA alterations, specifically HPA hypo-functionality. There was also no evidence that abused subjects had long term deficits in growth or growth processes. Future work will continue to investigate behavior and physiology into adulthood and attempt to identify biological mechanisms that may underlie the behavioral changes seen in abused subjects.

INDEX WORDS: maternal abuse, early adversity, stress, HPA axis, cortisol, growth, aggression, anxiety

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CHAPTER 1

INTRODUCTION

Early adverse experiences can lead to: physiological alterations (Cicchetti & Rogosch, 2001a; 2001b; De Bellis et al., 1994; De Bellis et al., 1999a; Heim, Ehlert, Hellhammer, 2000; Money, Annecillo, & Kelley, 1983; Olivian, 2003; Pine, 2003; Yehuda, Halligan, & Grossman, 2001;), behavioral problems (Ashman, Dawson, Panagiotides, Yamada, & Wilkinson, 2002; Flinn & England, 1995; Hardie, Moss, Vanyukov, Yao, & Kirillovac, 2002), and increase vulnerability to the development of psychiatric disorders (Cicchetti & Rogosch, 2001b; Cohen, Brown, & Smailes, 2001; Yehuda et al., 2001). Children, who have experienced maltreatment by their caregivers in the form of neglect, physical or sexual abuse, are particularly vulnerable (Cicchetti & Rogosch, 2001a; 2001b). Although there are many biological systems, impacted by the experience of early adversity, that provide mechanisms for the negative outcomes of childhood maltreatment, the hypothalamic-pituitary-adrenal (HPA) axis is considered a major contributor. The HPA axis is a major neuroendocrine system that is activated in response to stressful stimuli, such as those experienced by maltreated infants, and acts to direct behavioral, emotional, and physiological responses to stressors (Charmandari, Kino, & Chrousos, 2004; Johnson, Kamiliaris, Chrousos, & Gold, 1992; Sapolsky, Romero, & Munck, 2000; Raison & Miller, 2003; Selye, 1956). Chronic activation of the HPA axis during maltreatment in infancy may result in long-term alterations of the developing organism, including permanent HPA and behavioral alterations, and disruption of growth and metabolic processes.

The Early Social Environment as a Regulator for Biological Systems

According to Bowlby (1969), the attachment to primary caregivers serves to give the infant a sense of security and safety. Through interactions with the caregiver, the infant develops cognitive schemas, which enable the infant to make predictions about the environment and allow it to regulate emotions and behavior (Bowlby, 1969). Psychobiological theory proposes that interactions between the infant and caregiver are also critical to the development of biological systems, such as those activated by environmental stressors. Early in life, infants are not equipped to deal with environmental demands through cognitive or behavioral methods, and must deal with stressors on a physiological level (Kraemer, 1992). Therefore when the caregiver does not shield the infant from stressors in the environment, such as in the case of neglect or if the caregiver is the source of the stress, as is the case in physical or sexual abuse, the biological stress system of the infant is impacted and could become permanently altered. Changes in biological stress systems, such as the HPA axis, could in turn result in behavioral, cognitive, and emotional alterations (De Bellis, 2001).

Stress and the HPA Axis

The ability to respond appropriately to threats in the environment is critical to the survival of the organism (Walker, Welberg, & Plotsky, 2002). Some of the main neuroendocrine responses to stressors are mediated through the HPA axis. In response to stressful stimuli, corticotrophin releasing factor (CRF) is released by neurons in from the paraventricular nucleus (PVN) of the hypothalamus, and transported in the portal blood to the anterior pituitary where it stimulates the production and release of adrenocorticotropic hormone (ACTH) from the anterior pituitary (Chalmers, Lovenberg, & De Souza, 1995). ACTH is released into the systemic

circulation and stimulates the release of glucocorticoids (cortisol in primates, corticosterone in rodents) from the adrenal gland. (Herman & Cullinan, 1997; Herman et al., 2003).

The mature HPA axis exhibits a circadian rhythm with a peak around the time of waking and a decline throughout the day, with a trough during the quiescent time of the activity cycle. Superimposed upon this diurnal pattern of basal secretion is the activation of stressor-specific pathways that converge in the hypothalamus, where this information is integrated in the PVN neurons expressing CRF (Reul, & de Kloet, 1985). Maintenance of optimal basal and stress-induced levels of glucocorticoids is important for proper function not only of the HPA axis, but of many other biological systems. This regulation is mediated through a negative feedback system, whereby glucocorticoids bind to corticosteroid receptors in the pituitary, PVN and extra hypothalamic limbic regions to inhibit further release of ACTH and CRF. The ultimate reduction of glucocorticoid release serves to maintain the basal tone of the HPA axis and to restrain stress-induced HPA activation once the stressor is terminated. (Cullinan, Herman, Helmreich, & Watson, 1995; Herman & Cullinan, 1997; Herman et al., 2003).

Dysregulation of the system due to early adversity can result in permanent changes in the HPA axis, such as hyper-cortisolism, which involves increased basal and stress-induced secretion of glucocorticoid (Sapolsky, Krey, & McEwen, 1986) or HPA axis hypo-functionality, which can include decreased basal glucocorticoid availability due to a deficit in the production and release at the level of the adrenal cortex or decreased release of glucocorticoid secretagogues, CRF and/or ACTH, reduced cortisol response to stressors, or enhanced negative feedback (Gunnar & Vazquez, 2001; Heim, Newport, Bonsall, Miller, & Nemroff., 2000; Raison & Miller, 2003).

Early Adversity and HPA Dysregulation

There is substantial evidence that disrupting the normal infant-caregiver relationship in humans impacts the HPA axis (De Bellis, 2001; De Bellis et al., 1994; De Bellis et al., 1999a; Heim et al., 2000; Pine, 2003; Yehuda et al., 2001; Cicchetti & Rogosch, 2001a; 2001b). Changes in HPA regulation commonly accompany maltreatment; however the particular characteristics of the HPA dysregulation seem to depend on many variables, including the behavioral profile of the maltreated child and the time since maltreatment.

There is some evidence that during or near the time of abuse, maltreated children have a hyper-functioning HPA axis. Maltreated children with depression (De Bellis et al., 1994), post-traumatic stress disorder (De Bellis et al., 1999), and internalizing symptoms (e.g. anxiety, negative mood, withdrawal, and fear) (Cicchetti & Rogosch, 2001a) had elevated basal cortisol levels compared to controls. HPA axis hyper-functioning has also been discovered at the level of the pituitary. Abused children with depression that were still undergoing adversity showed an increased ACTH response to CRF administration (Kaufman et al., 1997). There is also evidence of hypo-functioning of the HPA axis. For example, maltreated children with depression do not show the normal afternoon decline in cortisol levels due to reduced morning basal levels (Hart, Gunnar, & Cicchetti, 1996; Kaufmann, 1991). Maltreated children, assessed as more behaviorally reactive, (Hart, Gunnar, & Cicchetti, 1995) and maltreated girls with externalizing symptoms (e.g. aggression and antisocial behavior) (Cicchetti & Rogosch, 2001b) also had lower morning basal levels of cortisol. Interestingly, maltreated girls with comorbid externalizing and internalizing symptoms demonstrated morning cortisol levels between those of girls with externalizing and girls with internalizing symptoms (Cicchetti & Rogosch, 2001b). These studies

not only indicate HPA dysregulation in maltreated children, but also demonstrate the association between childhood psychiatric disorders and HPA dysregulation.

Studies that have investigated the long-term effects of child maltreatment have done so primarily through the use of psychological and pharmacological challenges. Similar to the findings of HPA activity of maltreated children, the HPA activity of adults with a history of child abuse often varies depending on psychiatric diagnosis. Through the use of challenges, there is some evidence of HPA hypo-functionality in adults with a history of child maltreatment. Super suppression of cortisol or enhanced negative feedback in response to the administration of the synthetic glucocorticoid, dexamethasone, has been demonstrated in women with a history of maltreatment. The enhanced negative feedback was accompanied by an increase of lymphocyte GR receptor density, which indicates up-regulation, possibly due to sensitization of GR receptors from chronic cortisol release in childhood (Stein, Yehuda, Koverola, & Hanna, 1987). Adults with a history of childhood abuse and clinical depression also showed blunted ACTH levels in response to CRF administration. Abused women without depression showed lower levels of cortisol prior to and after the administration of ACTH. However the abused women without depression showed an increased ACTH response to CRF administration. Evidence of a hyper-functioning HPA axis has also been demonstrated in response to a psychological stressor. Both depressed and non-depressed abused women had higher cortisol and ACTH responses to a psychosocial stressor compared to non-maltreated women (Heim et al., 2001).

The Impact of Abuse on Behavior

The HPA axis is responsible for regulating a variety of physiological and behavioral processes, such as activating key brain areas necessary for assessment and attention to threatening stimuli (Charmandari, Kino, & Chrousos, 2004; Johnson, Kamiliaris, Chrousos, &

Gold, 1992; Sapolsky, Romero, & Munck, 2000; Raison & Miller, 2003; Selye, 1956). If the HPA axis is hyper or hypo-functioning, this could result in impaired reactions to environmental stimuli, such as extreme fear and aggression (Gunnar & Vazquez, 2006). Therefore it is not surprising that maltreatment, which is associated with changes in HPA regulation is also associated with behavioral problems and psychiatric disorders (Ashman et al., 2002; Flinn & England, 1995; Hardie et al., 2002) (Cicchetti & Rogosch, 2001b; Cohen et al., 2001; Yehuda et al., 2001).

Common behavioral outcomes of child maltreatment are anxiety, negative mood, social withdrawal, and fear, which are often referred to as internalizing symptoms (Bolger & Patterson, 2001; Cohen et al., 2001; Keiley, Howe, Dodge, Bates, & Petit, 2001; Manly, Kim, Rogosch, & Cicchetti, 2001; Young, Abelson, Curtis, Neese, 1997). However, symptoms often vary depending on the type of abuse. Although neglected (Manly et al., 2001) and sexually abused children (Bolger & Patterson, 2001) have been characterized as having elevations in internalizing symptoms, physically abused children were not found to differ with regards to internalizing behavior from non-maltreated children.(Manly et al., 2001). In adulthood however, physical abuse does seem to be related to internalizing symptoms. Of those adults diagnosed with anxiety disorders, 32% suffered neglect, 31% experienced physical abuse, and 15% were sexually abused as children (Young et al., 1997). Timing of the insult also seems to moderate internalizing symptoms. Children maltreated in early childhood (prior to age 5) had more internalizing symptoms than those abused later in life (between 6 and 9) and non-abused children (Keiley et al., 2001).

Another well-established outcome of childhood maltreatment is externalizing symptoms, such as aggression and antisocial behavior, which are common symptoms associated with

conduct disorder, and oppositional defiant disorder (see Cohen et al., 2001 for review). In a study of boys exposed to physical, sexual, or emotional abuse or neglect, maltreated boys had a higher prevalence of aggression, fighting, violence, and delinquency compared to non-maltreated boys (Stouthamer-Loeber et al., 2001). Maltreated children have also been characterized as more aggressive, less cooperative, more disruptive, and more withdrawn. Unlike physical neglect, which was associated with internalizing symptoms, physical abuse was associated with externalizing symptoms and aggression. Physically abused children were also more reactive and impulsive (Manly et al., 2001). Deficits in social information processing may underlie these externalizing symptoms. For example, physically abused children have been shown to be hyper-vigilant to aggressive stimuli, are more likely to believe others will behave aggressively toward them, and are more likely to respond aggressively when confronted with ambiguous social situations (Dodge, Pettit, Bates, & Valente, 1995; Dodge, Lochman, ,Harnish, Bates, & Pettit., 1997; Price & Glad, 2003).

Possibly as a result of the behavioral problems associated with maltreatment, maltreated children often have difficulty developing relationships with peers. Research indicates that maltreated children are more withdrawn and isolated when socializing with peers (Kaufman & Cicchetti, 1989) and that peers are more likely to avoid or reject maltreated children (Rogosch & Cicchetti, 1994). Maltreated children also rated themselves as less popular than other children (Wodarski et al., 1990) and peers rated maltreated children as less well-liked than other children (Bolger, Patterson, & Kupersmidt, 1998). Although some social deficits have been detected, other research has found that maltreated children have higher levels of friendship quality and more reciprocated playmates than non-maltreated children. However, friendship quality of chronically physically abused children declined from 2nd grade to 6th grade, ending up lower than

non-maltreated children. It has been hypothesized that maltreated children seek comfort in peers, and that they are competent enough to form relationships early in life, but as relationships become more complex, maltreated children do not have the social skills necessary to maintain positive peer relationships (Bolger et al., 1998).

The Impact of Abuse on Growth and Growth Processes

In addition to HPA functioning and behavioral alterations, early adverse experience can also impact growth processes. Growth failure in children living in stressful environments has been demonstrated in several studies (Albanese et al., 1994; Charmandari, Kino, Souvatzoglou, & Chrousos, 2003; Ellis, Fisher, & Zaharie, 2004; Pears & Fisher, 2005; Skuse, Albanese, Stanhope, Gilmour, & Voss, 1996). In humans, psychosocial dwarfism, which is characterized by lower than normal height and body weight, delayed onset of puberty, and delayed cognitive and social development (Johnson et al., 1992) and failure to thrive, which is based solely on retarded growth rate are documented conditions associated with infant maltreatment (Kerr et al., 2000; Money et al., 1983; Olivian, 2003).

Growth failure brought on by physical or emotional abuse or neglect is believed to be due, in part to, the effects of chronic activation of the HPA axis on growth processes. The activity of the growth hormone system is influenced under times of stress through the activity of the HPA axis. During chronic stress, glucocorticoids inhibit the release of growth hormone (GH) and growth hormone releasing hormones as well as decrease sensitivity of tissue to the effects of GH and additional growth factors stimulated by GH (Charmandari et al., 2003; Johnson et al., 1992; Wehrenberg, Janowski, Piering, Culler, & Jones, 1990). This idea is substantiated by the finding that children with psychosocial dwarfism show decreased GH levels when they are in the abusive or neglectful environments, but once they are placed into safe and nurturing

environments, the GH levels return to normal (Albanese et al., 1994; Skuse et al., 1996). A study of non-human primates demonstrated that parental care during infancy can have persistent effects on growth processes. In a study of the common marmoset (*Callithrix jacchus jacchus*), the frequency that the infant was carried and groomed during the first 3 weeks of life was positively associated with body weight during the juvenile period prior to puberty. Furthermore, infants that experienced abusive parenting (tail biting resulting in serious wounds) during the first 2-3 weeks of life had lower body weights as prepubescent juveniles and smaller knee-heel length and head-tail length around the time of puberty than non-abused infants (Johnson, Kamilaris, Calogero, Gold, & Chrousos, 1996).

Non-Human Primate Model of Infant Maltreatment

According to Maestripieri (1999), 2-10% of rhesus and pigtail macaque infants in large, captive settings are abused or neglected by their mothers. Infant abuse includes violent behavior, such as hitting, biting, dragging, and throwing (Troisi & D'Amato, 1983). The spontaneous occurrence of this abuse and neglect has provided the opportunity to investigate the physiological and behavioral effects of maternal abuse in non-human primates. The subjects in this study were rhesus macaques (*Macaca mulatta*), abused by their mothers during their first 3 months of life and non-abused rhesus monkeys. Previous data from these subjects has demonstrated the immediate effects of maltreatment on HPA axis regulation and behavior. Compared to control animals, maltreated subjects showed higher morning basal levels of cortisol at month 1, when abuse rates were highest, but showed a blunted ACTH response to CRF administration at 6 and 12 months of age (McCormack et al., 2003). Since early maltreatment and the chronic cortisol release during month 1 was followed by down-regulation of the HPA axis early in life, it is possible the HPA axis may remain hypo-functional later in life.

The current study continued to follow the subjects into the juvenile period. The study of this developmental time period is important to follow since it includes the onset of puberty, which is characterized by significant elevations in gonadal hormones that may increase vulnerability to stressors (Kirschbaum Kudielka, Gaab, Schommer, & Hellhammer, 1988; Mc Ewen, 2001; Spear, 2000). It is also a time when the incidence of anxiety and mood disorders increases (Born, Shea, & Steiner 2002; Forbes, Williamson, Ryan, & Dahl, 2004), particularly in children with previous exposure to adversity (Gunnar & Vazquez, 2006). Significant changes in affiliative behavior (Ehardt & Bernstein, 1987) and aggressive behavior (Bernstein & Ehardt, 1985) also occur during this time period in rhesus monkeys. Growth rates can also be measured at this time since adult skeletal maturation is not complete, until approximately 6 years of age in the rhesus monkey (Wilson & Tanner, 1991).

This study assessed the persistent effects of maternal abuse on physiology and behavior using a nonhuman primate model. It was hypothesized that the chronic stress of maltreatment early in life, which was associated with increased activation of the HPA axis during the first month of life (McCormack et al., 2003), would lead to suppressed HPA function in the abused juveniles. It was also predicted that HPA axis hypo-functionality would be accompanied by changes in social behavior, behavioral responses to stressors, growth and growth processes.

Hypotheses tested:

1. **Abused subjects would show a pattern of HPA hypofunctionality, compared to control subjects. These alterations would be manifested in one or more of the following ways:**

Basal HPA activity

- Abused subjects would exhibit lower basal plasma levels of morning cortisol compared to controls at 24, 30, and 36 months of age.

Basal HPA activity across the day (diurnal rhythm)

- The morning to afternoon and/or afternoon to night decrease in cortisol secretion would be blunted in abused subjects compared to controls at 24, 30, and 36 months of age, resulting in a flattened rhythm of diurnal cortisol secretion.

Negative feedback of glucocorticoids

- Negative feedback would be enhanced in abused subjects. Abused subjects would exhibit greater magnitudes of cortisol suppression in response to dexamethasone than control subjects at 24, 30 and 36 months of age.

Pituitary and adrenal functionality

- The pituitary and adrenal responses to the CRF challenge would be reduced in abused subjects compared to control subjects. Overall ACTH and cortisol responses to the CRF challenge would be blunted in the abused group compared to the control group at 24, 30, and 36 months of age.
- The adrenal responses to the ACTH challenge would also be reduced in the abused subjects compared to control subjects. Overall cortisol responses to ACTH would be lower in the abused subjects, in comparison to control subjects at 24, 30, and 36 months of age.

Decreased HPA stress response

- Abused subjects would have lower cortisol and/or ACTH levels in response to psychological stressors compared to control subjects.

2. **Abused subjects would demonstrate retardation in growth processes that would be manifested in any of the following ways:**

Physical growth

- Abused subjects would exhibit reductions in physical growth, as demonstrated by smaller height (crown to heel length, crown to rump length), delays in bone maturity, and lower bone mineral content, body weight, fat mass, and lean mass compared to control subjects at 36, 42, and 48 months of age.
- Abused subjects would exhibit reduced physical growth rate, as demonstrated by less changes in the above growth measurements from 36 months to 48 months.

Growth Hormone

- Abused subjects would demonstrate lower levels of growth hormone than control subjects at 36, 42, and 48 months of age.

Growth and HPA associations

- Growth hormone secretion would be positively associated with the previously described physical growth measures. Both growth hormone secretion and physical growth measures would be inversely associated with morning basal cortisol levels during month 1.

3. **Abused subjects and control subjects would have different behavioral responses when exposed to novel stimuli than control subjects at 24 months of age. Behavioral alterations were predicted to include any of the following:**

a) Abused subjects would show more inspection/manipulation, impulsivity, and aggression when exposed to novel stimuli than control subjects at 24 months of age.

- Abused subjects would inspect, manipulate, and bite novel objects more, show a shorter latency to inspect objects and would show more environmental exploration than control subjects.
- Abused subjects would show a shorter latency to touch, and bite novel objects than control subjects (impulsivity).
- Abused subjects would display more aggressive behavior (cage agitation, threat, slap, and bite objects) than control subjects.

b) When exposed to novel stimuli, abused subjects would show more fearful, defensive, and nervous behavior compared to control subjects at 24 months of age.

- Abused subjects would show more fearful and defensive, (crouch freeze, withdrawal, avoid, grimace, squeal, coo) and nervous (yawn, scratch, body shake) behavior than control subjects.

4. Abused subjects and control subjects would have different behavioral responses to aggression from social group members. Behavioral responses would include any of the following:

a) Abused subjects would display more aggressive behavior. Abused subjects would also show more retaliation and escalated aggression than control subjects.

- Abused subjects would show higher rates of aggressive behavior than control subjects.
- Abused subjects would be more likely to respond to aggression with aggression than control subjects (retaliation).

- Abused subjects would show more extreme forms of aggression (i.e. contact aggression) in response to less extreme forms of aggression (i.e. non-contact aggression) than control subjects (escalated aggression).

b) Abused subjects would show more defensive, nervous, and submissive behavior in response to aggression than control subjects.

- Abused subjects would show more submissive (lipsmack and present), nervous (scratch, yawn, body shake), and defensive behavior (crouch freeze, grimace, squeal, flee) in response to aggression than control subjects.

5. Abused subjects would show deficits in social behavior.

- Abused subjects would exhibit less affiliative behavior and would be in contact and proximity with other animals less frequently than control subjects.
- Abused subjects would be in proximity and contact with fewer social partners than control subjects.

CHAPTER 2

METHODS

Subjects and Housing

This study was conducted at the Yerkes National Primate Research Center Field Station, in Lawrenceville (Georgia). The subjects were 20 rhesus macaques (*Macaca mulatta*) born between March 19, 2002 and July 15, 2002. Subjects were housed in social groups (see Appendix A for group compositions) in a semi-naturalistic environment, consisting of outdoor compounds, measuring 38 x 38m with adjacent indoor housing. Water was available *ad libitum* and monkey chow was provided twice daily. Fresh fruit and enrichment items were provided once per day.

Ten of the subjects (6 females, 4 males) were offspring of abusive mothers and 10 subjects (6 females, 4 males) were offspring of non-abusive mothers. Prior to this study, infants were classified as abused if the mother was observed exhibiting at least 3 instances of the following violent behaviors towards the infant during its first 3 months of life: (1) dragging: the mother dragged her infant by its tail or leg while walking or running; (2) crushing: the mother pushed her infant against the ground with both hands; (3) throwing: the mother threw her infant with one hand while standing or walking; (4) stepping or sitting on: the mother stepped on her infant with one foot or both feet, or sat on her infant; (5) rough grooming: the mother restrained the infant and pulled her infant's hair causing distress calls. (6) abusive carry: mother carried infant with one arm away from her body (definition and inclusion criteria adapted from Maestripieri & Carroll, 1998; Maestripieri, Jovanovic, & Gouzoules, 2000; Troisi & D'Amato,

1983). Each abused infant was matched with a non-abused infant based on the following criteria: age (within one month), sex, and, when possible, social group and mother's dominance rank (see Appendix B). In December of 2003, one control female and one control male were dropped from the study. The control female was permanently removed from her social group for failure to maintain her weight, and the abused male died as a result of tetanus. Each subject was replaced by a sex, age, and maltreatment status-matched subject in January of 2004. In June of 2005, an abused female subject was euthanized due to chronic inflammatory illness (degenerative joint disease in both knees). In November of 2005 a control male and an abused female were removed from their larger social groups due to colony management decisions (in one case, the subject belonged to the highest ranking family, which had to be removed from the social group because they were severely attacking other animals; in the other case, the move was due to dominance hierarchy shifts that challenged the survival of the subject's family). The removed subjects were relocated to smaller indoor/outdoor enclosures with 7-8 family members. The enclosures include an indoor area measuring, 3.05 x 3.05 x 1.83m and an outdoor area measuring 3.05 x 2.15 x 1.83m. Seven out of eleven females (5 controls, 2 abused) were pregnant around 48 months of age.

Procedures

The studies described in this section were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and have been approved by the Emory University Institutional Animal Care and Use Committee (IACUC).

Training and Capture

Prior to this study, infants and their mothers were captured once per month during the first 6 months of life and every 6 months thereafter to obtain blood samples and to undergo

testing. All mothers had been previously trained to move on command from the outdoor enclosure into the indoor area, where they could be transferred into a squeeze cage via a transfer box. After they gave birth, mothers would run into the indoor enclosure carrying their infants for testing. Once in the squeeze cage, the infant was removed from the mother and sampling began on each animal. After 12 months, subjects were trained to go into the indoor area without their mothers and to present their legs for blood sampling and drug injections.

Blood Sampling

Blood samples were taken from the saphenous vein without anesthesia unless otherwise specified (see Challenge Procedures for exceptions). Basal blood samples were collected within 5-10 minutes of entering the building. All blood samples were collected with a 3 ml syringe. Samples consisted of 1 -2 ml of blood, which were collected in pre-chilled polypropylene tubes containing EDTA and immediately placed on ice. Plasma was separated by centrifugation at 3000 rpm for 12-15 minutes at 4ºC, then aliquoted and stored at -80ºC until assayed.

Blood Sample Assays

Plasma concentrations of cortisol were measured in duplicate 10µl aliquots by radioimmunoassay (RIA) using commercially available kits (Diagnostic Systems Laboratories, DSL, Webster, TX). Plasma ACTH levels were determined in duplicate 200 µl aliquots by a two-site radioimmunometric method using commercial kits by immunoradiometric assay (IRMA) (Nichols Institute Diagnostics, San Juan Capistrano, CA). Plasma concentrations of growth hormone (GH) were assayed by ELISA (Diagnostic Systems Laboratories, DSL, Webster, TX).

HPA Axis Function: Diurnal Rhythm of Cortisol Secretion (Basal)

Basal blood samples were collected at three different time points across the day, following published protocols (Sanchez et al., 2005): early morning (at sunrise), afternoon (midpoint between the morning and night samples), and night (1 hr after sunset), at 24, 30, and 36 months of age. Since the animals were living under natural lighting conditions, time points were selected from sunrise and sunset times charts published by the U.S. Naval Observatory for Lawrenceville (GA) in order to use the daylight, and not the clock time, as a reference for the diurnal samples and any other HPA axis-related sample. Blood samples were collected during the same season for all experimental groups, to control for circannual differences in HPA axis activity. Only one blood sample per week was collected per subject following a counterbalanced design for order of morning, afternoon, and night samples. Morning samples were always collected prior to the animals being fed to avoid meal-induced HPA axis activation.

HPA Axis Function: Pharmacological Challenges

1. Dexamethasone suppression test

The dexamethasone (DEX) challenge test was conducted at 24, 30, and 36 months. Subjects were captured at night and a basal blood sample was taken. Subjects remained in the indoor enclosure until given a dexamethasone sodium phosphate injection (0.25 mg DEX/kg body weight, i.m) and then were returned to the social group. Injection time was calculated as 10 hours before the following day's sunrise. The following day a basal blood sample was taken at sunrise (10 hours after DEX injection) and another in the afternoon (16 h after DEX injection).

2. CRF and ACTH challenges

The CRF and ACTH challenge tests were conducted at 24, 30, and 36 months. Subjects were captured at sunrise, immediately anesthetized (5 mg telazol/kg body weight, i.m.), and a

basal blood sample was taken (0 min). An i.v. bolus of r/h CRF (50 μg CRF/kg body weight), human ACTH (1-24) (1 μg ACTH/kg body weight) or a vehicle solution (10 mM acetic acid/sterile 0.9% saline) was administered into the saphenous vein of the animals. Additional blood samples (1 ml) were taken from the femoral vein at 15, 30 and 60 min after the i.v. infusion, to analyze ACTH and/or cortisol responses to each drug. Only one pharmacological challenge was done per week, following a counterbalanced design for drug order. Ketamine supplements (5 mg/kg body weight, i.m.) were administered to keep animals sedated when necessary to complete testing. Upon recovery each subject was returned to its social group.

HPA Axis Function: Responses to Stressors

Two ml of blood were collected (see blood sampling procedures) at the end (32 min) of the stress tests described below when subjects were approximately 24 months of age (see Stress Induced Behavior: Laboratory Paradigms procedures).

Growth Measurements: Physical Growth

Physical growth measurements were collected at 36, 42, and 48 months of age following published protocols (Wilson et al., 2003). Subjects were sedated with telazol (5 mg/kg body weight, i.m.), followed with Ketamine supplements (5 mg/kg body weight, i.m.) to keep animals sedated when necessary. Once anesthetized, the subject was weighed then placed on its side on an examination table. To measure the subject's crown-rump length, the back and neck were aligned and one end of the Vernier calipers was placed at the top of the head and the other caliper arm at the ischial callosities. To measure the subject's height, the animal was placed on its back, with the head and legs straight. With the leg stretched tight, one arm of the caliper was placed at the top of the head and the other at the left heel. All measurements were taken in centimeters. To ensure accuracy, each measurement was conducted twice and averaged.

Immediately following the body length measurements (subjects were still sedated), body composition (percentage of body lean, fat, and bone mass) was assessed using dual energy x-ray absorptiometry (DEXA, Norland XR-26 Mark II, Norland Instruments). For this, the subjects were placed on the scanner in the supine position. The legs were flexed, and the legs and arms were taped to secure the position.

Immediately following the DEXA scan (subjects still anesthetized), an X-ray of the hands and wrists was taken (Model SCD-105, Kraemer Corporation). From the X-rays, skeletal maturation was rated following the radius-ulna-short bones option of the Tanner-Whitehouse-2 scoring system (Tanner et al., 1983), previously modified and used for rhesus monkeys (Wilson et al., 2003). The method involves the assignment of differential weights (0-8) based on bone thickness to the radius, ulna and bones of the hand (first, third, and fifth metacarpals; first proximal phalanx; and third and fifth middle and terminal phalanges). Radius and ulna bone scores were doubled and added to scores of the other bones (Wilson, Gordon, Rudman, & Tanner, 1988; Wilson & Tanner, 1991). The scoring was conducted by one examiner who was blind to the abuse status of the subjects.

Growth Measurements: Growth Hormone Plasma Levels

Samples were collected using a previously validated approach to quantify developmental changes in GH levels of rhesus macaques (Wilson, et al., 2003). Two blood samples, 30 minutes apart, were collected in the morning (1 hour after sunrise) and at night during complete darkness (1 hour after the end of astronomical twilight). This procedure was replicated the following week. The night samples collected each week were assayed for growth hormone and averaged for analysis. The night blood samples were used for the GH analysis since GH secretion peaks at

night in rhesus monkeys (Wilson, Chikazawa, Fisher, Mook, Gould, 2004). The additional samples were kept frozen to use for future analyses.

Stress-Induced Behavior: Laboratory Paradigms

Laboratory paradigms were conducted when the subjects were approximately 24 months of age. One week prior to testing, subjects were habituated to the laboratory environment by exposing each subject to the testing room and apparatus (squeeze cage with plexi-glass box attached) 3 times per week for 20 minutes sessions. During habituation, a jelly bean was placed on top of the testing box ("baited"), but no objects were placed inside of the testing box, so that subjects learned that there was a positive reward available for approaching the testing apparatus. For all habituation and testing sessions, subjects were run into the inside enclosure from their outside compound, transferred into a squeeze cage via a transport box and transported to the testing room. A digital camera was set up approximately 1.5 meters from the squeeze cage to videotape the subjects during testing. The order of the assessment tests were counterbalanced to control for paradigm order effects. All testing sessions were separated by at least 3 days.

1. Approach/Avoidance Paradigms (Neutral/Positive and Fear-Evoking Objects)

Once inside the test cage, a clear plexi-glass testing box (30 x 30 x 30 cm) was attached to the outside of the cage. Subjects were able to reach through a small hole in the test cage to reach items in the box, but items were not small enough to fit through the hole. During two separate sessions, either 6 neutral/positive or 6 fear-evoking objects were presented to the subject in the testing box, with a jelly bean on the top of the box. The approach/avoidance paradigm was designed to measure the conflict between exploratory behaviors and behavioral inhibition in response to non-familiar objects of varying threatening intensities (Bethea et al., 2004; Williamson et al., 2003). To choose fear-evoking objects, objects that had been shown to evoke

fear in previous studies (Bethea et al., 2004; Williamson et al., 2003) were first presented to and tested with monkeys that were not the subjects of this study. Based on this test, objects that elicited defensive responses or fear (see Table 1 for behavioral ethogram) were chosen as fear-evoking objects and those that did not were chosen as neutral or positive objects. For the neutral object test, a roll of construction tape, a rubber ball, a piece of kiwi, a plastic cup, a toy train, and a baby rattle were presented in the order listed. During the fear object test, subjects were presented with a toy frog with large eyes, a toy dinosaur with large teeth, a stuffed pig that moved and "oinked", a plastic owl with large eyes accompanied by predatory hawk calls, a rubber snake, and a mirror (to simulate the presence of a social intruder), in the order listed. For the presentation of the mirror, the testing box was removed from the cage and the mirror was attached to the right side of the cage with clips. For both the neutral and fear object tests, there was 2 minutes of habituation, followed by the presentation of each object for 5 minutes (total duration of test: 32 minutes).

2. Human Intruder Paradigm

The human intruder paradigm was used to assess fearful and anxious behavior to an unfamiliar human intruder under different conditions: non-threatening profile condition (no eye contact) and a threatening stare condition (human makes direct eye contact with the monkey). Subjects were run into the inside enclosure from their outside compound and transferred into a squeeze cage (test cage) in the testing room using a transport box. The subjects were placed in the testing cage for 10 minutes (alone condition). An unfamiliar female experimenter ("human intruder") entered the building and stood next to the digital camera, with her profile to the subject. After the 10 minute profile condition, the human intruder left the building and returned

after 2 minutes. Upon returning to the building, the intruder stood in the same spot as in the profile condition, but made direct eye contact with the subject (stare condition) for 10 minutes.

Immediately following each laboratory paradigm, 2 ml of blood was collected from the subject's saphenous vein for measurements of ACTH and cortisol plasma responses to the tests. Sessions were videotaped for behavioral measures. All of the videotapes were scored for measurements of locomotor activity, vocalizations, nervous behavior (scratching, self-grooming, body shake, yawn), fearful and defensive behavior (e.g., grimace, freezing behavior), aggressive behavior and submissive behavior (i.e. lipsmack and present). Latency to manipulate, inspect, and bite the object, manipulation, sniffing, biting, and slapping of the objects, and exploration of the testing box were also scored for the approach/avoidance paradigms (see Table 1 for ethogram). Prior to the beginning of data scoring, the percentage of agreement between observers exceeded 90%.

Social Behavior

Observations were conducted starting around the 4th year of life (approximately 48 months). Data was collected between 0700 and 1100 h when the social group was locked out of the inside enclosure. Subjects were observed in their home compounds from observation towers. A total of 5, 1 hour duration focal observations were conducted on each subject. Subject order was randomized.

Aggressive behavior and responses to aggressive behavior were recorded as frequency data. Aggressive behavior directed toward the subject or that the subject directed toward other group members was recorded. Aggressive behavior was defined as threat, chase, grab, slap, pin, and bite. Responses of the subject to aggressive episodes were also recorded. Submissive behavior (lipsmack and present), defensive behavior (squeal, flee, grimace, and crouch freeze),

nervous behavior (scratch, body shake, and yawn), and aggressive behavior (threat, chase, grab, slap, pin, and bite) (see Table 1 for ethogram) were recorded. Sex and age category (i.e. infant, juvenile or adult) of the aggressors were also recorded.

Every 10 minutes during the focal observation, an instantaneous scan sample was conducted. At the instant of the scan, the observer recorded how many group members the subject was in contact with and how many members the subject was in proximity with (see Table 1 for ethogram). The percentage of agreement between observers exceeded 90% prior to the beginning of data collection.

Table 1: Behavioral ethogram for laboratory paradigms and social group observations

Behavior	Operational Definition	Scored For
Fearful/Defensive		
Crouch Freeze	Subject has tense posture with ventrum pressed down to or towards the bottom of the cage. Arms and legs are pressed against the body. Remains for 3 secs. New bout after 3 sec interruption when coded as frequency. Frequency for social behavior (SB). Duration for (approach avoidance) AA.	AA/HI/SB*
Avoid	Subject is facing the object/ intruder with tense body posture and makes quick shifts in gaze, avoiding eye contact with object/intruder or subject is stationary with back turned away from object/intruder. Must remain for 3 seconds. Duration.	AA/HI
Withdrawal	Subject makes a quick, jerky motion to the back of the cage, away from the object/intruder. Frequency	AA/HI
Grimace	Subject pulls back lips to expose clenched teeth. New bout after 3 sec interruption. Frequency.	AA/HI/SB
Coo	Vocalization made by rounding and pursing the lips. New bout after 3 sec interruption. Frequency.	AA/HI
Squeal	High pitched vocalization. New bout after 3 sec interruption. Frequency.	AA/HI
Flee	Subject runs away from an individual that is running towards the subject. New bout after 3 sec interruption. Frequency.	SB
Nervous		
Yawn	Mouth open wide with teeth exposed. Frequency.	AA/HI/SB
Scratch	Subject draws its fingernails or toenails across its fur. New bout after 3 sec interruption. Frequency.	AA/HI/SB
Body Shake	Shaking of head and shoulder region of the body, like dog shaking. Frequency	AA/HI/SB
Aggression		
Cage Agitation	Subject slaps, bites, or shakes cage. Duration.	AA/HI

Behavior	Operational Definition	Scored For
Threat	Includes **open mouth** (staring with mouth and eyes wide open), **bob** (head moves up and down or side to side with direct eye contact, **lunge** (subject makes quick forward movement in slightly crouched position with direct eye contact, and **raised eyebrow** (direct eye contact with eyes wide open, without open mouth). New bout after 3 sec interruption. Frequency	AA/HI/SB
Chase	Individual runs toward a subject that is running away. Includes visual fixation on the subject that is fleeing. New bout after 3 sec interruption. Frequency.	SB
Grab	Individual closes hand around a subject and vigorously pulls hand toward self without letting go. Frequency.	SB
Slap	Vigorous contact with a subject with open hand. Frequency.	SB
Pin	Use of both hands to hold and push the subject into the ground. Frequency.	SB
Bite	Common usage. Duration and Latency to for AA. Frequency for SB.	AA/SB
Submissive		
Lipsmack	Quick movement of jaw pressing lips together. Lips open and close and tongue protrudes in and out. New bout after 3 seconds interruption. Duration for AA. Frequency for SB.	AA/HI/SB
Present	Subject presents rear to animal/object/ intruder. Subject is slightly crouched with knees in locked position. Frequency.	AA/HI/SB
Exploration		
Cage Explore	Oral, tactile, or visual exploration (at least 3 seconds) of the cage (no biting, slapping, or shaking cage and not moving around cage). Duration.	AA/HI
Box Explore	Oral, tactile, or visual exploration of the box. Includes reaching into box. Object Inspect/Manipulate take precedence over Box Explore. Duration.	AA
Object/Intruder Inspect	Subject is watching (visual fixation) object or intruder for at least 3 seconds. Duration and Latency to.	AA/HI

Behavior	Operational Definition	Scored For
Object manipulate	Subject manipulates object by touching object or grabbing the object (not vigorous and can be maintained for long period of time). Duration and Latency to.	AA
Slap Object	Subject makes brief and vigorous contact with object with hand. Frequency.	AA
Eat fruit/jelly bean	Subject puts fruit or jelly bean in mouth and chews. Latency to.	AA
Other		
Locomotion	Any self-induced change in location of self. Includes changes in location through walking, climbing, rolling, hopping, bouncing, and dropping from ceiling to floor (no repetitive pattern). Duration	AA/HI
Proximity	Subject is within arm's reach of another animal. Scored as occurrence or non-occurrence. Number of animals in proximity also recorded.	SB
Contact	Subject approaches and sits, stands, or lies while having body contact with an animal. Scored as occurrence or non-occurrence. Number of animals in proximity also recorded.	SB

AA = Approach/Avoidance Paradigm. HI = Human Intruder Paradigm. SB = Social Behavior Observations.

Data Analysis

Data was checked for normality and homogeneity of variance before running statistical analyses. Repeated measures ANOVA analyses were conducted on data where repeated measures were taken. Dominance rank was added as a covariate when it significantly correlated with measurements. Post-hoc analyses of significant interactions were performed with Bonferroni-corrected t-tests. For analysis involving comparisons between two groups, such as between control and abused subjects, Mann-Whitney U analyses were conducted due to small sample sizes and violations of normality. Chi-square analyses were performed on categorical data, and proportions tests were used when investigating differences between 2 independent proportions. Area under the curve with respect to ground was calculated using the trapezoid rule and consisted of the measurements obtained at specific time points and the time distance between the measurements. For data collected when females were pregnant of after they gave birth (i.e. growth measurements at 48 months and social behavior), Mann-Whitney U analyses were conducted to determine if there were differences in dependent variables based on pregnancy. When pregnancy effects were detected, the variable was dropped from the analysis due to the confounding effect of pregnancy. Alpha levels were set at 0.05, and one-tailed p values were reported for analyses conducted on directional hypotheses. Proportions tests were calculated using VassarStats (© Richard Lowery, Vassar College, Poughkeepsie, NY) and lag sequential analyses were conducted with Noldus Observer, version 5.0. The remaining data were analyzed using SPSS, version 9.0.

Physiological Data

With the exception of area under the curve data, physiological data (HPA axis data and growth hormone data) were analyzed using repeated measures ANOVAs. For the diurnal rhythm

of cortisol analyses, cortisol levels indicating possible stress levels, rather than basal levels (> 5 standard deviations from the mean), were removed from the analyses. For all challenge data, cortisol and ACTH levels outside of the normal physiological range (cortisol levels above 50 ug/dl and ACTH levels above 500 ng/ml) and therefore suggestive of assay problems were excluded from the analyses. For the growth measurements analyses, the data from 48 months was dropped from the analysis due to the confounding effect of pregnancy on the growth variables.

Behavioral Data

For the laboratory paradigm behavioral data, complex and simple contrast analyses were first conducted to determine if behavioral differences occurred across the 6 neutral or 6 fear objects. When no significant differences were found, behavioral data was collapsed across the 6 objects for each test for further behavioral analyses. Otherwise, separate analyses were conducted for each object. Due to the low occurrence of behavior in some behavioral categories, unless the behavior was performed by all animals, behavioral data was transformed from durations and frequencies to categorical data (yes or no) based on whether or not each animal performed the behavior. Categorical data was analyzed using Chi-square and reported as likelihood ratios with Fisher's exact p values or Breslow-Day values. When the behavior was performed by all animals, between-subject ANOVAs were performed on the duration or frequency data.

For social group behavior, lag sequential analyses were conducted to determine the subjects' aggressive (grab, slap, pin, bite, threat, chase), nervous (yawn, scratch, body shake), and defensive responses (crouch freeze, grimace, squeal, flee) within 10 seconds of aggression received. Submissive responses (lipsmack, present) were not included in the analyses due to low

occurrence. The analysis for aggression followed by nervous behavior was excluded due to the effect of pregnancy on this variable. Mann-Whitney U analyses and proportions tests were conducted on social behavior data.

CHAPTER 3

RESULTS

Stress-Induced Behavior: Laboratory Paradigms

For the neutral object test there were significant group (control, abused) effects for exploration of the testing cage ($F(1, 15) = 6.07$, $p = 0.03$), visual inspection of the neutral objects ($F(1, 15) = 4.60$, $p = 0.049$), and avoidance of the neutral objects ($\chi^2 = 5.3$, $p = 0.04$). Abused animals ($M = 0.50$, *S.E.M* = ±0.05) spent a greater proportion of time exploring the cage than control animals ($M = 0.34$, *S.E.M.* = ±0.05) (see Figure 1). Abused animals ($M = 0.13$, *S.E.M* = ±0.06)) also spent more time inspecting the neutral objects than control animals ($M = 0.07$, *S.E.M.* = ±0.015) (see Figure 2). Chi-square analyses revealed that a smaller percentage of abused subjects (20%) avoided the neutral objects compared to control subjects (70%) (see Figure 3). Chi-square analyses also showed a group by sex effect for exploration of the testing box during neutral object 4 (blue cup) ($\chi^2 = 4.14$, $p = 0.042$). Post-hoc analyses revealed that a larger percentage of abused males (75%) explored the box compared to control males (0%) ($\chi^2 = 11.90$, $p < 0.01$) (see Figure 4).

For the fear object test, there was a group effect for the latency to inspect fear objects ($F(1, 15) = 6.32$, $p = 0.02$) and for biting the fear objects ($\chi^2 = 5.3$, $p = 0.04$). Abused animals ($M = 6.7$, *S.E.M.* = ±5.12) had a shorter latency to inspect the fear objects than control animals ($M = 35.03$, *S.E.M.* = ±10.89) (see Figure 5). A larger percentage of abused animals (80%) bit the fear objects compared to control animals (30%) (see Figure 6). There were group by sex effects for avoiding object 2 (dinosaur) ($\chi^2 = 6.15$, $p = 0.01$; see Figure 7) and for slapping object 4 (owl) (χ

^{2} = 4.62, p = 0.03; see Figure 8). However, post-hoc analyses did not reveal differences between control and abused females (χ^2= 1.55, p = 0.27) or between control and abused males (χ^2 = 6.09, p = 0.07).

A group effect was found for displacement behavior during the stare condition of the intruder test (χ^2= 4.54, p = 0.05), with a larger percentage of abused subjects (77.78%) showing displacement behavior (yawn, scratch, and body shake) compared to control subjects (30%) (see Figure 9).

No group or group by sex effects were found for freezing, withdrawal, grimace, coo, squeal, flee, cage agitation, threat, chase, grab, lipsmack, present, manipulate object, or locomotion during the neutral objects tests or the human intruder paradigm (p > 0.05).

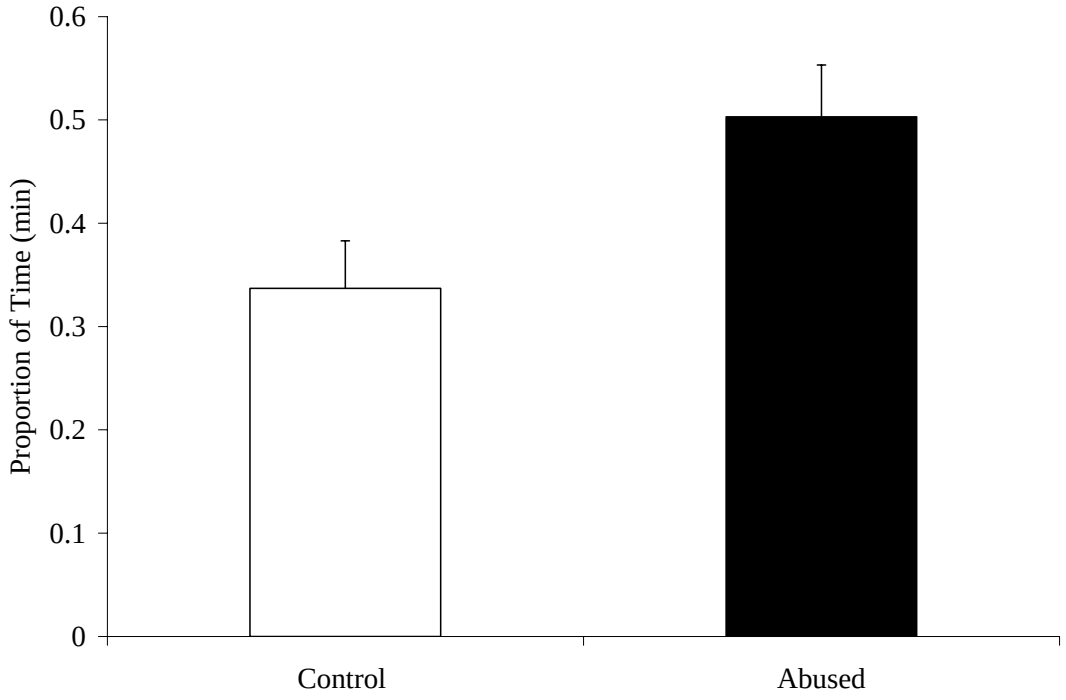

Figure 1: Proportion of time spent exploring during the neutral object test

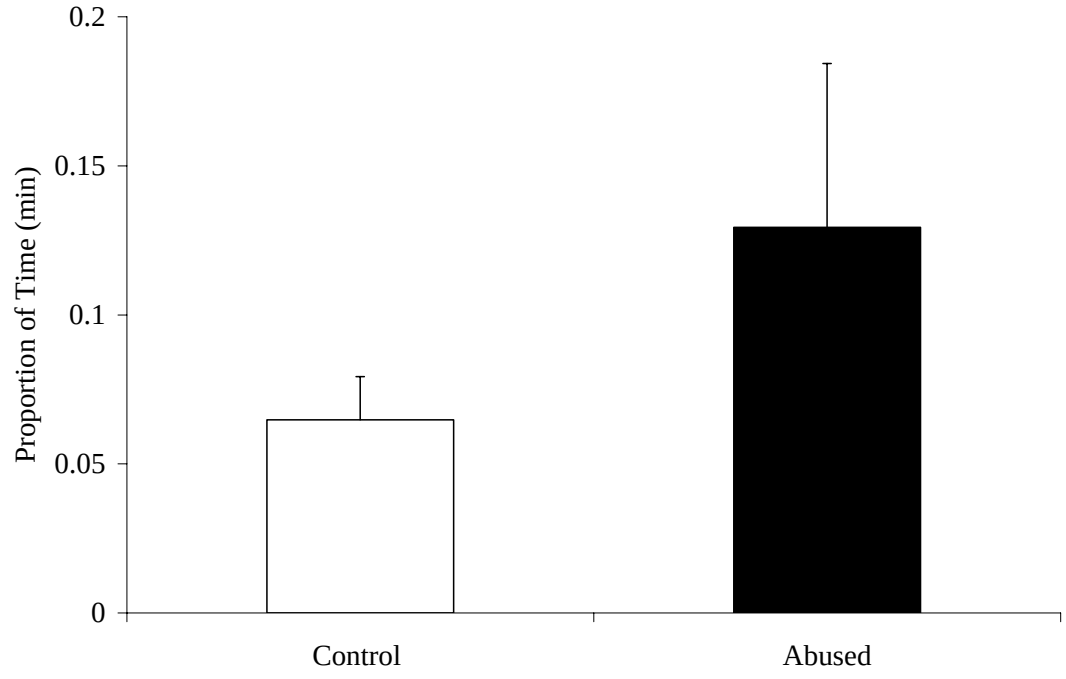

Figure 2: Proportion of time spent inspecting the neutral objects

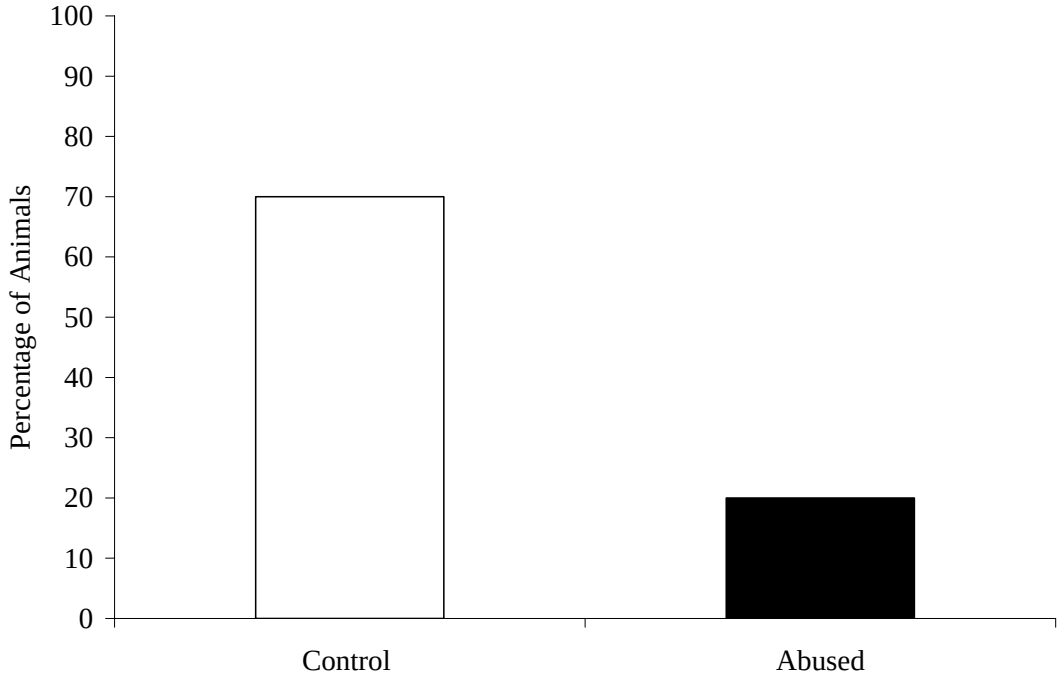

Figure 3: Percentage of subjects that avoided the neutral objects

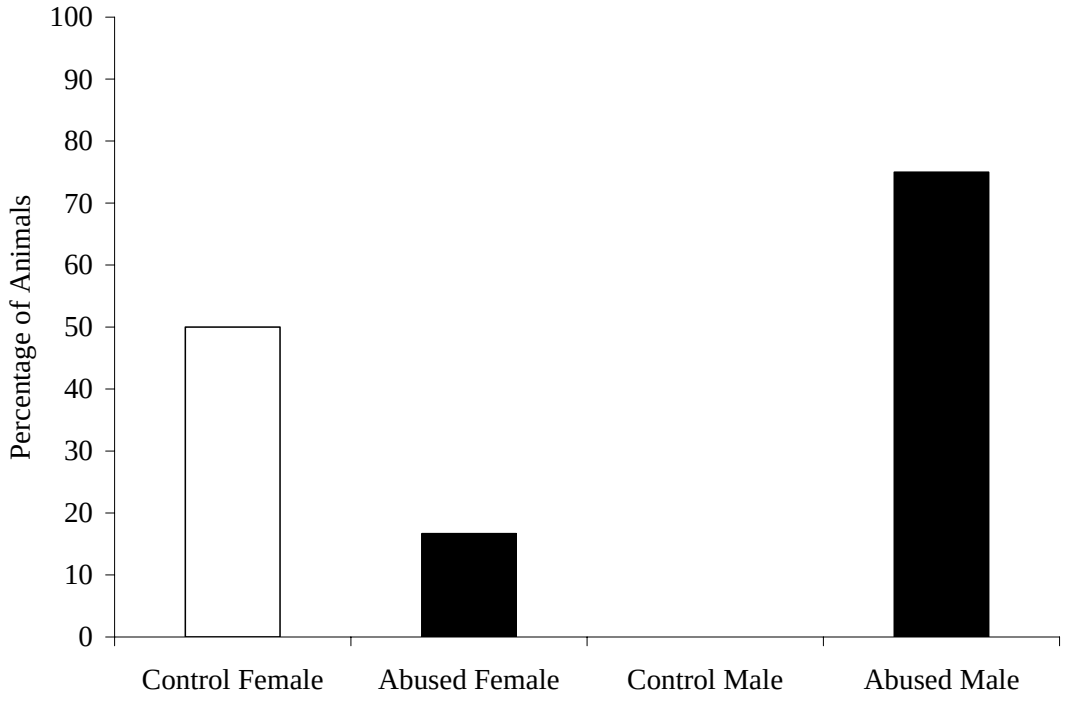

Figure 4: Percentage of subjects that explored the testing box during the neutral object test

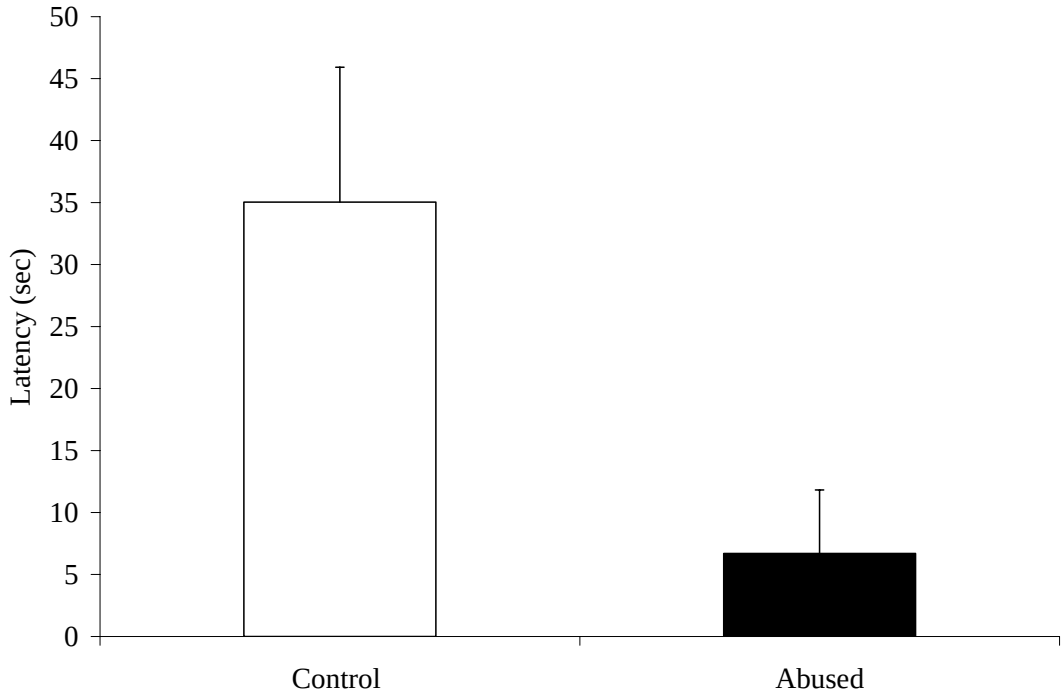

Figure 5: Latency to inspect fear objects

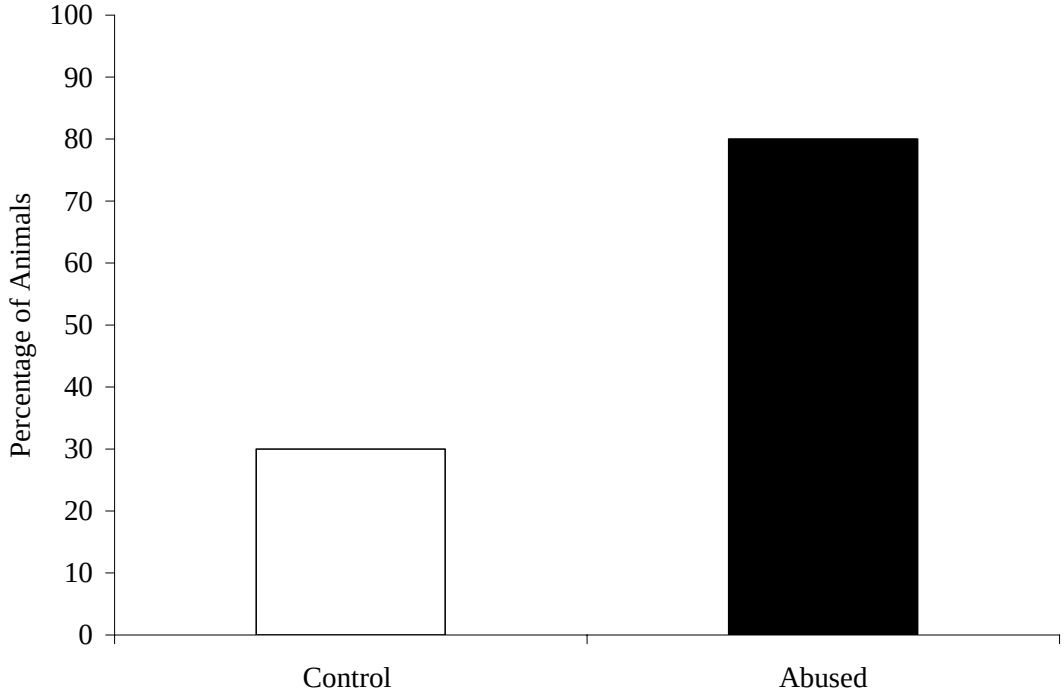

Figure 6: Percentage of subjects that bit fear objects

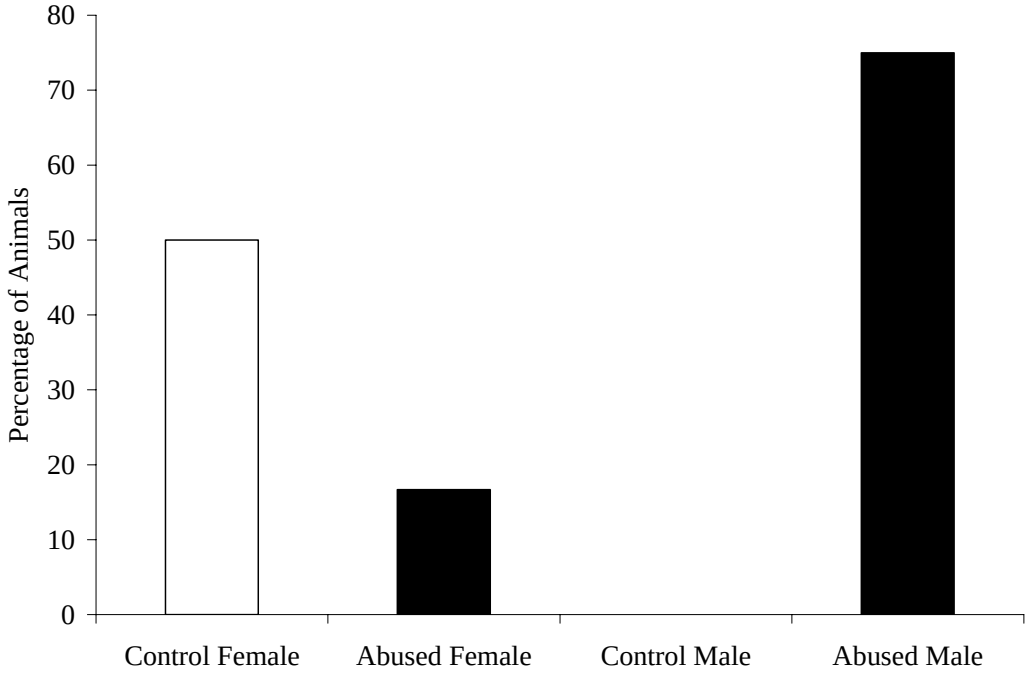

Figure 7: Percentage of subjects that avoided fear object 2 (dinosaur)

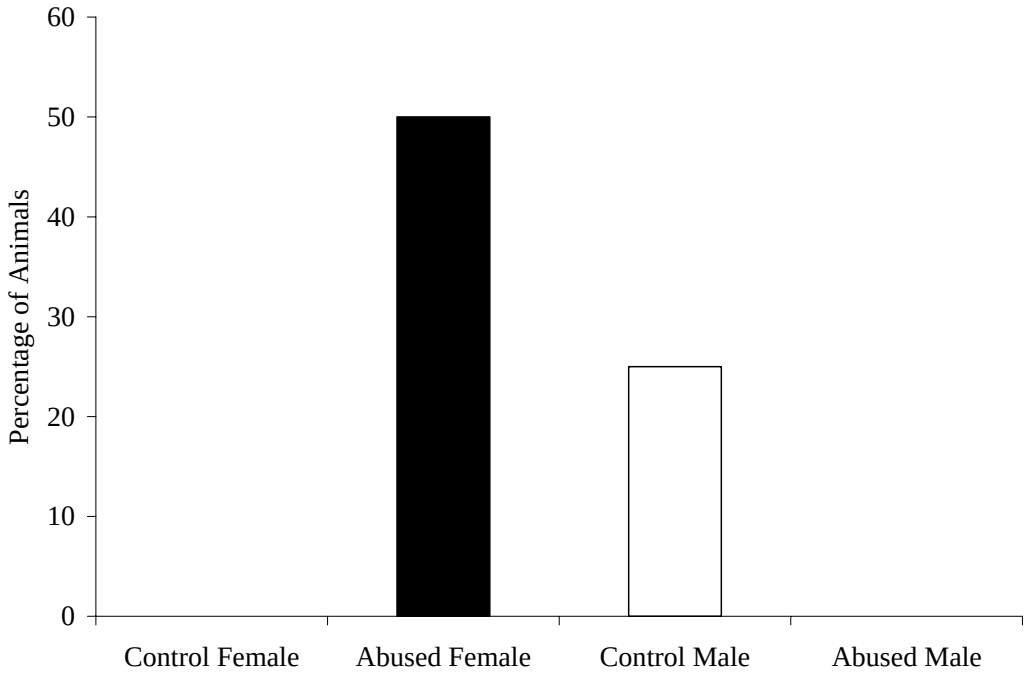

Figure 8: Percentage of subjects that slapped fear object 4 (owl)

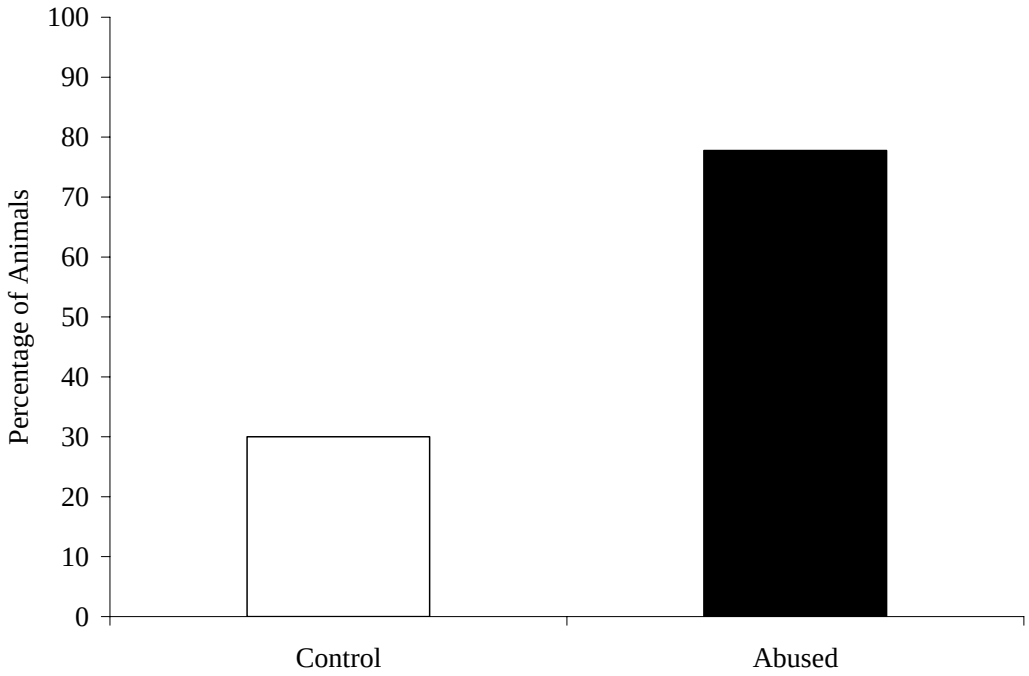

Figure 9: Percentage of subjects that displayed nervous behavior during the stare condition of the human intruder paradigm

Social Behavior

Group members did not display more aggression to abused subjects (M = 6.09, *S.E.M.* = ±1.32) than to control subjects (M = 4.86, *S.E.M.* = ±0.67) (z = 0.24, p = 0.81) (see Figure 10). However, abused subjects did display more aggression per hour (M = 15.27, *S.E.M.* = ± 2.92) than control subjects (M= 9.54, *S.E.M.* = ± 1.42) (z = 1.68, p = 0.047) (see Figure 11). Further analyses showed that abused subjects threatened, grabbed, and bit other animals more than controls subjects (z = 1.68, p = 0.047; z = 1.97, p = 0.02; z = 2.17, p = 0.02, respectively; see Table 2). Abused animals were not more likely to chase, slap, or pin other animals more than control animals (z = 1.54, p = 0.06; z = 0.53, p = 0.299; z = 0.16, p = 0.31, respectively; see Table 2).

Abused subjects were not more likely to respond to aggression with defensive behavior compared to control subjects (z = 0.88, p = 0.19). Abused animals were more likely to respond to aggression with aggression (i.e. retaliation) than control animals (z = 4.04, p = 0.04). Further analyses showed that abused animals were more likely than control animals to respond to contact aggression with non-contact aggression (z = 3.21, p = 0.0007), to respond to non-contact aggression with contact aggression (z = 2.76, p = 0.003), and to respond to non-contact aggression with non-contact aggression (z = 1.62, p = 0.05). Abused animals were not more likely than control animals to respond to contact aggression with contact aggression (z = 1.03, p = 0.15) (see Table 3).

Abused subjects (z = 2.73, p = 0.003) and control subjects (z = 2.91, p = 0.002) were less likely to show more contact aggression in response to non-contact aggression than in response to contact aggression (see Table 3). The percentage of change between the amount of contact aggression in response to contact aggression and the amount of contact aggression in response to

non-contact aggression did not differ between abused subjects (8.84% change) and control subjects (8.49% change) ($z = 1.28$, $p = 0.10$).

The proportion of time in contact and proximity with group members out of the total number of scans did not differ between control subjects (422/695 or 61%) and abused subjects (341/598 or 57%) ($z = 1.35$, $p = 0.09$). The average number of contact partners per scan did not differ between control subjects ($M = 1.70$, *S.E.M.* = ± 0.08) and abused subjects ($M = 1.73$, *S.E.M.* = ± 0.13) ($z = 0.72$, $p = 0.24$).

Table 2: Rates of aggression displayed per hour ± S.E.M.

Type of aggression displayed	Control	Abused
Non-contact aggression	5.14 ± 0.80	9.30 ± 2.56
Threaten	4.58 ± 0.75	8.10 ± 2.24*
Chase	0.56 ± 0.13	1.20 ± 0.35
Contact aggression	4.40 ± 0.70	5.97 ± 0.83
Grab	0.83 ± 0.19	1.47 ± 0.23*
Slap	2.73 ± 0.41	2.45 ± 0.46
Pin	0.09 ± 0.05	0.20 ± 0.07
Bite	0.75 ± 0.16	1.85 ± 0.34*

* Indicates significant difference between control and abused (Mann-Whitney U, $p < 0.05$)

Table 3: Proportion of aggression and defensive behavior displayed following aggression out of the total proportion of aggression received

Aggression received	Response displayed	Control proportion	Abused proportion
All aggression	All aggression*	59/352	132/456
All aggression	Defensive	112/352	132/456
Non-contact	Non-contact*	15/192	42/252
	Contact*	8/192	20/252
Contact	Non-contact*	14/158	34/155
	Contact	20/158	26/155

* Indicates significant difference between control and abused (proportions test, $p < 0.05$)

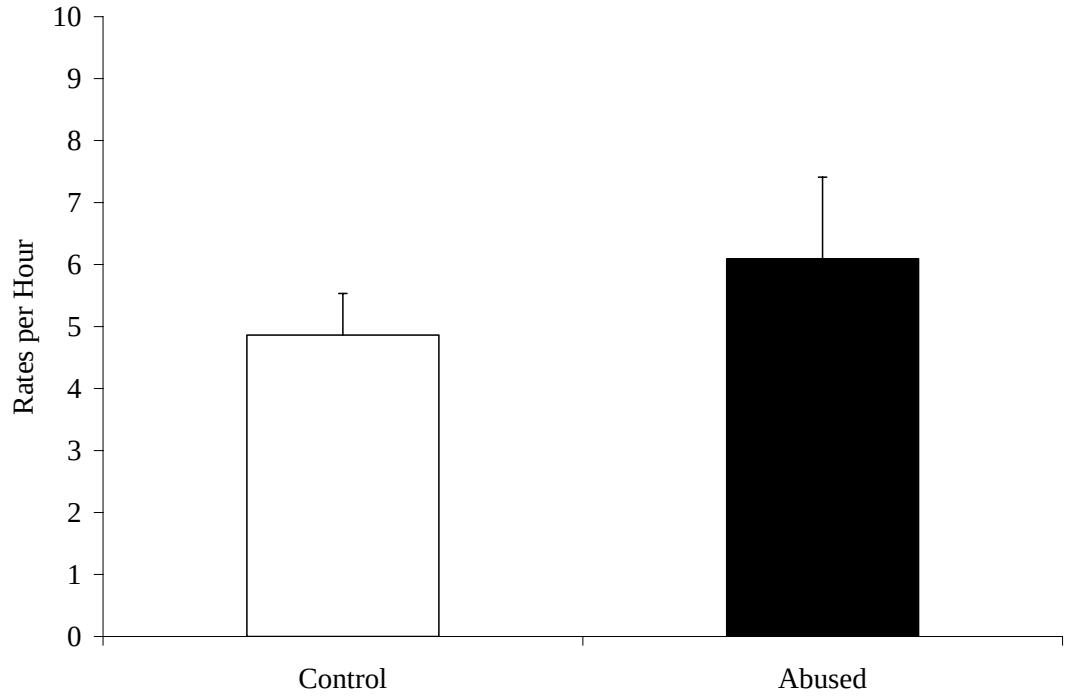

Figure 10: Total aggression received

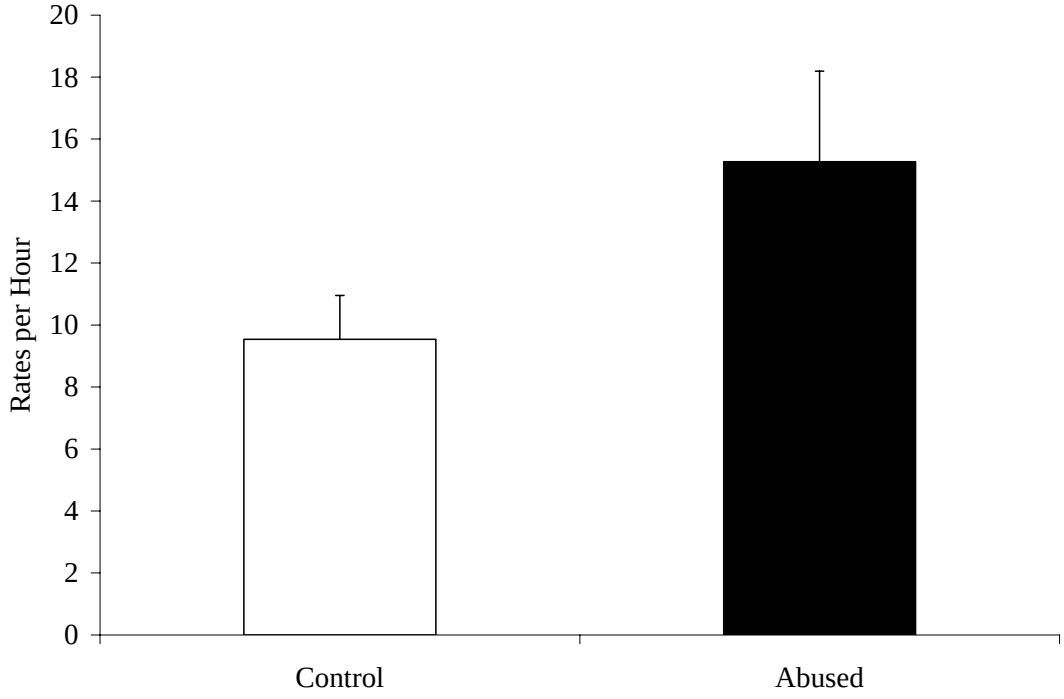

Figure 11: Total aggression displayed

HPA Axis Function

There were no group (control, abused) effects or group by time (morning, noon, and night) effects for basal cortisol levels at 24, 30, or 36 months of age ($p > 0.05$). Area under the curve cortisol values, which represented the total hormonal output throughout the day (morning, noon, night time points), did not differ between control and abused subjects at 24, 30, or 36 months ($p > 0.05$; see Figure 12).

There were no group (control, abused), group by time (morning or noon), group by condition (basal or dexamethasone treated), or group by time by condition effects for cortisol levels during the dexamethasone suppression test at 24, 30, or 36 months ($p > 0.05$; see Table 4).

Analyses for area under the curve values did not reveal significant effects for the CRF and ACTH challenge tests at 24, 30, or 36 months. There were no group by drug (vehicle, CRF) effects for cortisol or ACTH secretion from 15 minutes to 60 minutes post-CRF administration. There was also no significant group by drug (vehicle, ACTH) effect for cortisol secretion from 15 minutes to 60 minutes post-ACTH administration ($p > 0.05$; see Table 5).

There were no significant group (control, abused), group by paradigm (neutral object test, fear object test, human intruder paradigm), group by sex, or group by sex by paradigm effects for the cortisol (see Figure 13) and ACTH responses to the approach/avoidance or human intruder paradigms ($p > 0.05$).

Table 4: 24, 30, and 36 month basal and post-DEX morning and noon cortisol levels

		Morning		Noon	
		Basal	**DEX**	**Basal**	**DEX**
24 months	**Control**	16.63 ± 0.92	1.97 ± 0.84	12.35 ± 1.06	2.78 ± 1.33
	Abused	15.98 ± 0.86	3.04 ± 0.80	13.21 ± 1.01	4.42 ± 1.26
30 months	**Control**	14.27 ± 0.92	2.76 ± 0.98	11.23 ± 0.67	2.37 ± 1.13
	Abused	15.14 ± 0.972	3.97 ± 1.04	11.14 ± 0.71	5.36 ± 1.19
36 months	**Control**	15.45 ± 1.00	1.21 ± 0.26	10.61 ± 0.62	0.86 ± 0.52
	Abused	14.69 ± 1.06	1.50 ± 0.27	9.13 ± 0.65	1.69 ± 0.55

Table 5: 24, 30, and 36 month AUC cortisol and ACTH values following vehicle, ACTH, or CRF administration

		Cortisol AUC Values			ACTH AUC Values	
		ACTH Injection	**Vehicle Injection**	**CRF Injection**	**Vehicle Injection**	**CRF Injection**
24 months	**Control**	2.51 ± 0.14	1.40 ± 0.10	2.10 ± 0.11	4.10 ± 0.34	8.57 ± 1.55
	Abused	2.43 ± 0.12	1.58 ± 0.09	2.15 ± 0.15	4.41 ± 0.48	8.43 ± 1.15
30 months	**Control**	2.26 ± 0.11	1.53 ± 0.09	1.72 ± 0.10	3.99 ± 0.48	6.57 ± 0.88
	Abused	2.40 ± 0.13	1.45 ± 0.08	1.82 ± 0.14	3.76 ± 0.42	7.57 ± 1.01
36 months	**Control**	2.17 ± 0.13	1.39 ± 0.10	2.13 ± 0.19	3.44 ± 0.57	6.94 ± 1.24
	Abused	2.40 ± 0.14	1.47 ± 0.11	2.16 ± 0.18	3.69 ± 0.53	6.64 ± 1.23

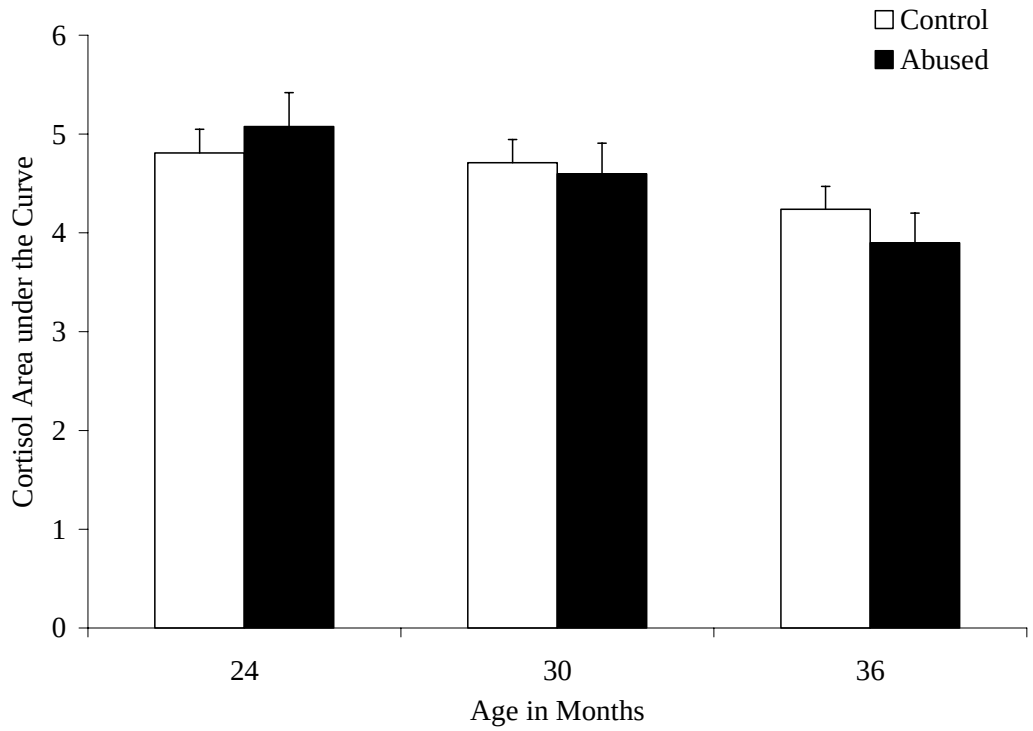

Figure 12: 24, 30, and 36 month total basal cortisol output throughout the day

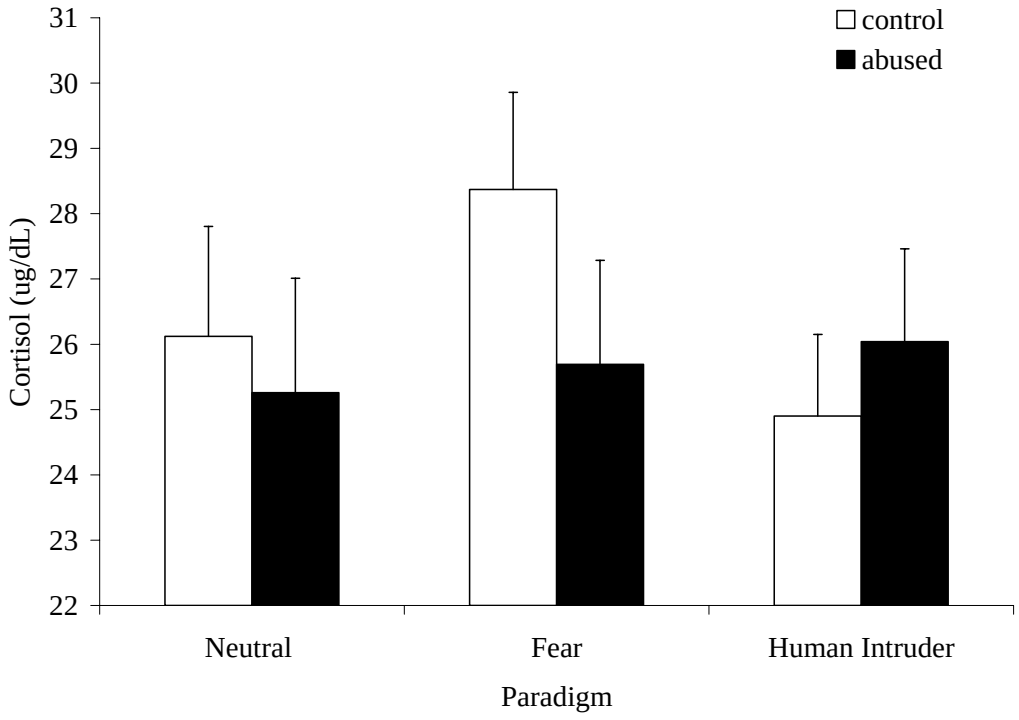

Figure 13: Cortisol levels following the laboratory paradigms

Growth Measurements

There was a group (control, abused) by age (36 months, 42 months) by sex effect for bone maturity ratings ($F(1, 14) = 4.71$, $p = 0.048$). However, post-hoc tests of the interaction effect did not reveal significant differences between control and abused females at 36 or 42 months respectively ($t(10) = 2.00$, $p = 0.07$; $t(9) = 0.12$, $p = 0.91$) or between control and abused males at 36 or 42 months respectively ($t(3.8) = 1.55$, $p = 0.20$; $t(6) = 0.30$, $p = 0.76$ see Table 6). Post-hoc tests also did not reveal a significant difference between control and abused males and females at 36 months or 42 months respectively ($t(18) = 0.240$, $p = 0.63$; $t(17) = 0.02$, $p = 0.89$). An age effect for bone maturity ratings revealed that ratings were higher at 42 months compared to 36 months of age ($F(1, 15) = 84.36$, $p < 0.001$), and a sex effect for bone maturity ratings revealed that females had higher ratings than males ($F(1, 14) = 5.24$, $p < 0.04$) There were no group, group by sex, or group by sex by age effects for crown-rump or crown-heel length, bone mineral content (see Table 6), body weight lean or fat mass or growth hormone levels (see Table 7). There were no significant correlations between growth hormone levels and physical growth measurements or between cortisol levels at month 1 and growth measurements ($p > 0.05$).

Table 6: 36 and 42 month body length, bone mineral content, and bone maturity ratings

			Crown-Rump Length	Crown-Heel Length	Bone Mineral Content	Bone Maturity Rating
Group x Sex x Age Effects						
Females	**36 months**	**Control**	44.96 ± 0.55	68.83 ± 1.37	197.95 ± 8.70	71.00 ± 1.29
		Abused	43.88 ± 0.60	68.58 ± 1.06	198.93 ± 8.04	74.50 ± 1.18
	42 months	**Control**	46.33 ± 0.86	72.76 ± 1.40	234.47 ± 13.95	78.33 ± 1.45
		Abused	46.34 ± 0.69	73.22 ± 1.45	222.76 ± 10.86	78.60 ± 1.69
Males	**36 months**	**Control**	46.74 ± 0.54	72.08 ± 0.40	230.95 ± 17.90	71.75 ± 2.56
		Abused	45.79 ± 0.59	70.10 ± 0.38	210.13 ± 5.22	67.50 ± 0.96
	42 months	**Control**	49.43 ± 0.92	77.14 ± 1.52	258.20 ± 23.25	76.25 ± 1.32
		Abused	48.2 ± 0.77	73.86 ± 0.76	262.45 ± 15.12	75.75 ± 1.03*
Group Effects						
Both Sexes	**Both Ages**	**Control**	46.86 ± 0.43	72.70 ± 0.81	230.39 ± 8.78	74.33 ± 0.93
		Abused	46.14 ± 0.45	71.60 ± 0.84	224.26 ± 9.12	73.86 ± 0.96
Sex Effects						
Female	**Both Ages**	**Both Groups**	45.47 ± 0.41	71.01 ± 0.76	214.22 ± 8.23	75.38 ± 0.87
Male			47.58 ± 0.48*	73.29 ± 0.88	240.43 ± 9.61	72.81 ± 1.01*
Age Effects						
Both Sexes	**36 months**	**Both Groups**	45.43 ± 0.29	70.06 ± 0.54	210.18 ± 5.43	70.96 ± 0.75
	42 months		47.57 ± 0.42*	74.25 ± 0.66*	244.47 ± 7.93*	77.23 ± 0.75*

* Indicates significant effect

Table 7: 36 and 42 month body weight, lean mass, fat mass, and growth hormone levels

			Body Weight	Lean Mass	Fat Mass	Growth Hormone
Group x Sex x Age Effects						
Females	**36 months**	**Control**	4.55 ± 0.21	3784.50 ± 197.61	485.12 ± 47.24	3.65 ± 4.05
		Abused	4.45 ± 0.17	3703.83 ± 179.29	476.43 ± 75.42	9.05 ± 4.44
	42 months	**Control**	5.57 ± 0.28	4506.67 ±286.73	764.4 ± 117.84	5.07 ± 1.53
		Abused	5.33 ± 0.19	4470.40 ± 151.35	583.58 ± 74.95	4.09 ± 1.68
Males	**36 months**	**Control**	5.07 ± 0.24	4230.75 ± 208.18	553.93 ± 116.10	12.28 ± 5.67
		Abused	4.94 ± 0.16	4164.75 ± 190.65	519.45 ± 60.08	8.69 ± 7.82
	42 months	**Control**	6.42 ± 0.45	5547.75 ± 308.22	541.93 ±151.35	2.79 ± 0.74
		Abused	6.16 ± 0.38	5207 ± 301.84	631.83 ± 87.00	0.54 ± 0.22
Group Effects						
Both Sexes	**Both Ages**	**Control**	5.40 ± 0.18	4517.42 ± 150.28	586.34 ± 54.45	5.95 ± 1.75
		Abused	5.25 ± 0.18	4423.74 ± 156.18	539.28 ± 56.58	5.59 ± 1.81
Sex Effects						
Female	**Both Ages**	**Both Groups**	5.00 ± 0.17	4153.59 ± 140.98	563.84 ± 51.08	5.46 ± 1.64
Male			5.65 ± 0.19*	4787.56 ± 164.62	561.78 ± 59.64	6.07 ± 1.91
Age Effects						
Both Sexes	**36 months**	**Both Groups**	4.78 ± 0.10	4008.20 ± 93.60	495.19 ± 35.92	8.42 ± 2.31
	42 months		5.87 ± 0.16*	4932.95 ± 136.48*	630.43 ± 56.84*	3.12 ± 0.87*

* Indicates significant effect

CHAPTER 4

DISCUSSION

Although the evidence from this study does not indicate that maltreatment leads to long-term alterations in HPA axis function or alterations in growth or growth processes, there is some evidence that rhesus monkeys exposed to maternal maltreatment as infants have long-term behavioral alterations.

Social and Stress-Induced Behavior

As predicted, abused subjects displayed more nervous behavior than control subjects during the stare condition of the human intruder test. A common outcome of child maltreatment is internalizing problems, such as anxiety and depression (Bolger & Patterson, 2001; Cohen et al., 2001; Keiley et al., 2001; Manly et al., 2001; Flinn & England, 1995; Young et al., 1997), so the finding of higher rates of nervous behavior in the abused monkeys is compatible with what is seen in the human literature. This finding of increased anxiety has also been replicated using animal models of mother-infant relationship disruption (Caldji, Diorio, & Meaney, 2000; Caldji et al., 1998; Huot, Thrivikraman, Meaney, Plotsky, 2000; Kalinichev, Easterling, Plotsky, & Holtzman, 2002; Ladd, Huot, Thrivikraman, Nemeroff, & Plotsky, 2004; Levine, 1967; Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; Plotsky & Meaney, 1993; Sanchez et al., 2005). However, compared to control subjects, abused subjects did not display more nervous, defensive or fearful behavior during the approach avoidance paradigms. Because these emotion-related behaviors are context dependent (Buss, Davidson, Kalin, & Goldsmith, 2004), it is possible that a highly threatening condition was necessary to detect differences between control and abused

subjects for those particular behavioral categories. The stare condition of the human intruder test was designed to be the most intense threat out of all of the laboratory conditions. This may explain why differences in nervous, defensive, and fearful behavior between abused and control subjects were not detected during the other approach avoidance paradigms or during the profile condition of the human intruder task.

When exposed to neutral and fear objects during the approach/avoidance paradigms, abused subjects demonstrated more inspection than control subjects. When exposed to the neutral objects, abused subjects visually inspected both the testing environment and the objects more than control subjects. Contrary to my predictions, but compatible with the previous finding, abused subjects were also less likely to avoid the neutral objects than control subjects. During the fear object test, abused subjects inspected the objects more quickly than control subjects. Abused subjects also bit the fear objects more than control subjects, demonstrating more aggressive behavior toward the threatening objects than control subjects.

Abused subjects also displayed more aggressive behavior within their social groups. Even though abused subjects were not the recipients of more aggression by group members than control subjects, abused subjects displayed more total aggression than control subjects. The aggression displayed by abused subjects consisted of both non-contact and contact aggression. Abused subjects threatened, grabbed, and bit other animals more than control subjects while in their social groups. Responses to aggression also differed between abused and control subjects. Abused subjects were more likely to respond to aggression with aggression (i.e. retaliation) although abused subjects were not more likely to escalate aggressive episodes as predicted. Abused subjects demonstrated higher rates of aggression and aggression in response to

aggression, but they did not respond inappropriately to aggression by using a more extreme form of aggression than was necessary.

With regard to aggression, the abused subjects of this study resemble maltreated children. A common behavioral outcome seen in maltreated children is aggression (Dodge et al., 1995; Dodge et al., 1997; Manly et al., 2001; Price & Glad, 2003; Stouthamer-Loeber et al., 2001; Wodarski et al., 1990). The finding of greater inspection is also compatible with the human literature on childhood maltreatment. Physically abused children were found to attend to aggressive cues, attribute aggression to others, and respond aggressively to ambiguous stimuli more often than non-abused children (Dodge et al., 1995; Dodge et al., 1997; Price & Glad, 2003). It is possible that the aggression displayed by the abused subjects may be due in part to their increased attention to environmental stimuli. The increased attention may be a result of neuroanatomical changes as a result of maternal maltreatment. Early adverse experiences, such as abuse, have been hypothesized to influence brain circuits that mediate the HPA stress response and that are responsible for threat assessment. Key brain areas involved in the circuitry implicated in early adversity, HPA responses to stressors, and threat assessment are the amygdala, hippocampus, prefrontal cortex (Bremner, 2003; De Bellis et al., 1999b); Glaser, 2000; Kaufman, Plotsky, Nemeroff, & Charney, 2000; Pine, 2003; Teicher, Anderson, Polcari, Anderson, & Navalta, 2003). Future studies will be necessary to investigate if in fact neuroanatomical changes can be detected in these key brain areas through, for example, magnetic resonance imaging and diffusion tensor imaging.

Contrary to predictions, affiliative behavior did not differ between abused and control animals. Abused animals spent an equal amount of time in contract and proximity with group members and had an equal average number of contact and proximity partners compared to

control animals. Evidence in the human literature suggests that maltreated children have problematic peer relations. Maltreated children have been found to be more withdrawn and isolated when socializing with peers (Kaufman & Cicchetti, 1989) and less well-liked than other children (Bolger et al., 1998). Peers were also more likely to avoid or reject maltreated children (Rogosch & Cicchetti, 1994).However, other research has found that maltreated children have higher levels of friendship quality and more reciprocated playmates than non-maltreated children (Bolger et al., 1998). This study did not solely focus on peer relationships; affiliative behavior was based on proximity and contact between the subject and any group member other than the subject's own infant. Future research should focus on the quality of the relationships between the subjects and group members. By investigating affiliative behavior other than contact and proximity, such as grooming, and the animal that is responsible for initiating and maintaining contact, the question of relationship quality could be addressed.

HPA Axis Function

Contrary to predictions, abused subjects did not show a pattern of HPA axis hypo-functionality compared to abused subjects at 24, 30, or 36 months of age. Basal HPA activity did not differ between abused and control subjects. Specific predictions regarding diurnal HPA activity were that morning cortisol plasma levels would be lower in the abused subjects and that abused subjects would have a flattened rhythm of diurnal cortisol secretion. However, no group effects or group by time of day effects were detected for the basal cortisol levels. Total cortisol secretion throughout the day also did not differ between the control and abused subjects. Although abused subjects demonstrated higher morning basal levels of cortisol during the first month of life, there is no evidence that the abused subjects in this study had any long-term

alterations in their diurnal pattern of basal cortisol secretion. Therefore, it seems that the abused subjects recover from the HPA axis alterations detected at earlier ages (McCormack et al., 2003).

Also, contrary to predictions, abused subjects did not show alterations in the negative feedback of glucocorticoids. Maintenance of optimal basal and stress-induced levels of glucocorticoids is regulated via the negative feedback system, whereby glucocorticoids inhibit further release of CRF, ACTH, and cortisol. Therefore, it was believed one possible mechanism that could lead to hypocortisolism in abused subjects would be enhanced negative feedback in response to dexamethasone, a synthetic glucocorticoid. However, the cortisol responses to the dexamethasone were similarly inhibited in both control and abused subjects.

As indicated by the results of the ACTH and CRF challenge tests, differences in responsiveness at the level of the pituitary or the adrenal were not detected. Pituitary and adrenal responses to CRF were not blunted in abused subjects as was expected. Also contrary to predictions, adrenal responses to ACTH were not blunted in response to ACTH. Total hormonal output throughout the CRF and ACTH challenges did not differ between the control and abused subjects. Although abused subjects had blunted ACTH responses to CRF administration at 6 and 12 months of age, it seems that the abused subjects recovered from the previously demonstrated HPA alterations (McCormack et al., 2003).

Contrary to predictions, abused subjects did not have lower cortisol or ACTH responses to stressors than did control subjects. Cortisol and ACTH levels following the approach/avoidance and human intruder paradigms did not differ between control and abused subjects.

There is substantial evidence from both the human and animal literature that disrupting the normal infant-caregiver relationship impacts the HPA axis (Andrews & Rosenblum, 1991;

1994; Champoux, Coe, Schanberg, Kuhn, & Suomi,1989; Cicchetti & Rogosch, 2001a; 2001b; De Bellis, 2001; De Bellis et al., 1994; De Bellis et al., 1999a; Dettling et al., 2002a; 2002b; Heim et al., 2000; 2001; Johnson et al., 1996; Kaufman, 1991; Levine & Mody, 2003; Parker et al., 2004; Pine, 2003; Sanchez et al., 2005; Stanton et al., 1988; van Oers, de Kloet, & Levine, 1998; van Oers, de Kloet, Whelan, & Levine, 1998; van Oers, de Kloet, & Levine, 1999; Yehuda et al., 2001). My prediction that the abused subjects would have a hypo-functioning HPA axis was based on the theory that early adversity may induce frequent elevations in cortisol secretion that may result in down-regulation of the HPA axis or components of the HPA axis (Cicchetti & Rogosch, 2001a; 2001b; Gunnar & Vasquez, 2001; Hardie et al., 2002). Thus, subjects exposed to abuse would show reduced cortisol secretion, decreased pituitary and adrenal reactivity, or enhanced negative feedback. This theory is supported by the findings of a hyper-responsive HPA axis during the time of child maltreatment in humans (Kaufman et al., 1997), and a hypo-functioning HPA axis in adults abused as children (Heim et al, 2000; 2001). In this study, maternal abuse did seem to act as a chronic stressor, as evidenced by the fact that the abused subjects had higher morning basal cortisol levels than control subjects did during month 1, when abuse rates were highest. Subjects also demonstrated blunted ACTH responses to CRF at 6 and 12 months of age (McCormack et al, 2003). However, in the present study, there is no evidence that the chronic stress of maternal maltreatment resulted in a persistent down-regulation of any component of the HPA axis, suggesting that the abused subjects recover normal HPA axis function with age.

Methodological problems may have contributed to the inability to detect HPA alterations in the abused subjects. Conclusions regarding the diurnal pattern of cortisol secretion in the subjects were based on one sample per subject for each time point. Gunnar and Talge (in press)

propose that a single sample in humans may be a poor reflection of typical cortisol levels. Studies from their lab have found that in order to obtain a significant test-retest correlation, at least 3 cortisol samples are required However they also note that when environmental conditions are controlled about an hour prior to testing, test-retest cortisol levels are significantly related (Gunnar & Talge, in press). In the present study, an effort was made to ensure that there were no disturbances to the group, such as the veterinary staff entering the adjoining enclosure or severe group conflict prior to sampling. However, since the groups were not observed at all times, strict environmental control can not be assumed. The single sample cortisol and ACTH values were also used as comparison values in the analysis of the dexamethasone suppression test data. If the basal cortisol and ACTH values were not stable indices of the subjects' normal values, suppression in response to the dexamethasone in comparison to baseline could have been over or under-estimated.

Due to our priority to not stress the animals prior to the laboratory paradigms and the time necessary to set-up for them, blood samples within ten minutes of disturbing the group could not be collected. Therefore the cortisol and ACTH levels do not have a baseline comparison. This is not ideal, and it is possible that differences in responses to stressors were not detected because of differences in cortisol and ACTH levels prior to the start of the paradigms. However, the comparison of effects across the three paradigms would have detected differences in how abused and control subjects responded to the three stressors that varied in threatening intensity.

Growth Measurements

Contrary to predictions, rhesus macaques maternally abused as infants did not show growth impairments later in life. Although there was a significant age by group by sex effect for

bone maturity ratings, differences were not detected between control and abused females or between control and abused males at 36 or 42 months of age. The interaction may have been due to significant main effects of age and sex. Abused males and females also did not exhibit smaller height or lower body weight, percentage of body fat, or bone mineral content compared to control subjects at 36 or 42 months of age. Rates of growth between month 36 and month 42 also did not differ between abused and control subjects. Differences in growth hormone levels were also not detected between abused and control males and females at 36 or 42 months of age. Growth hormone levels did not positively correlate with physical growth measurements.

Previously we found that the abused subjects had higher morning basal cortisol levels than controls at month 1, when maternal abuse rates were highest (McCormack et al., 2003). Since the activity of the growth hormone system is influenced by stress through HPA activity (Charmandari et al., 2003; Johnson et al., 1992; Holboer, 1995; Wehrenberg et al., 1990), I predicted that increased cortisol early in life would be associated with reductions in physical growth later in life. However, cortisol levels at month 1 were not inversely correlated with physical growth measurements at months 36 and 42.

Previous studies have found long term growth alterations as a result of early life stressors (Johnson et al., 1996; Skuse et al., 1996). Impaired growth was detected in juvenile marmosets that were abused as infants although nearing adulthood, the correlation between early care and growth weakened (Johnson et al., 1996). In the human literature, a proportion of children exposed to early life stressors have growth hormone insufficiency and short stature that persisted even once the child was removed from the stressful environment (Pears & Fisher, 2005; Skuse et al., 1996). However, in the current study maltreatment and high cortisol levels early in life did not result in long term alterations in physical growth or physical growth processes.

Unfortunately, there is no growth data on the subjects between birth and 36 months, so it is impossible to know if maternal maltreatment and high cortisol levels early in life influenced growth processes prior to 36 months. Many children show spontaneous catch-up and reversibility of growth-hormone insufficiency when they are removed from the stressful circumstances (Albanese et al., 1994; Money et al., 1983; Olivian, 2003; Skuse et al., 1996). Since the timing of maternal maltreatment of the abused subjects was only during the first 3 months of life, it is possible that any alterations in growth or growth hormone were reversed by the time the subjects were measured as juveniles. It is also possible that since the growth hormone levels were based on only two samples, that the levels were not a true indicator of the subjects' normal growth hormone levels.

General Discussion

This study supported the hypothesis that maternally abused subjects would show increased aggression and greater inspection/exploration. Under highly threatening situations, abused subjects also show more nervous behavior than control subjects. This study did not show that maternal abuse caused long-term changes in the HPA axis function, or growth. One possibility for the null results is that the maternal abuse was not severe enough to cause long-term physiological changes or that it was not severe enough for all subjects. There was a large variation in the rates of abuse received during infancy. During the first 3 months, hourly rates of abuse ranged from 0.30 to 6.4, with an average rate of 1.65 abusive episodes per subject. It is also possible, since the subjects were not observed at all times during the first 3 months of life, that some of the control subjects may also have been abused. However, the mothers that were selected as control mothers have never been seen abusing their infants prior to or since this study. Also, the higher levels of cortisol of the abused subjects at month 1 during the time of

abuse, suggests that the abused subjects were experiencing a chronic stressor. Future studies should also consider the quality of maternal care received during infancy instead of solely abuse status. A median split of rejection rates by the mother during infancy, indicates that 22 % of control subjects and 67 % of abused subjects had mothers that rejected their infants at high rates. By focusing on a broader category of maltreatment that can include neglectful behavior, specific maternal stimuli necessary for proper physical and emotional development may be uncovered.

This study provided evidence that rhesus monkeys maternally abused as infants showed some behavioral alterations, but recover from the HPA axis alterations seen at earlier ages and do not show persistent growth alterations from 36-42 months of age. Abused subjects were more aggressive when exposed to stressful stimuli in the laboratory and when interacting with group members in their social groups. The abused subjects also showed greater attention when exposed to potentially threatening stressors in the laboratory. This may indicate differences in information processing that may mediate the relationship between maternal abuse and aggressive behavior.

This primate model of maternal maltreatment may provide a tool to investigate the biological mechanisms underlying the aggressive behavior seen in maltreated children. Since there were no differences found in HPA diurnal activity, negative feedback, pituitary or adrenal sensitivity, or responses to stressors between abused and non-abused subjects, future research should focus on other possible biological mechanisms that could be responsible for mediating the behavioral effects discovered in this study. Future research should focus on brain morphology and neurochemistry in order to identify biological mechanisms that contribute to the differences in attention and aggression that were found between abused and control subjects.

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APPENDIX A

SOCIAL GROUP COMPOSITIONS

Compound	Number of subjects	Total number of females	Total number of males	Total number of juveniles
A1	3	30	3	30
A2	6	49	2	104
A4	8	40	2	80
D1	3	41	2	59

APPENDIX B

LISTING OF ABUSED INFANTS AND THEIR MATCHED CONTROLS

Abused Infants	Sex	Compound	Control Infants	Sex	Compound
1a	F	A4	1c	F	A4
2a	F	A2	2c	F	A2
3a	F	D1	3c	F	D1
4a	F	A4	4c	F	A2
5a	F	A1	5c	F	A4
6a	F	D1	6c	F	A1
7a	M	A2	7c	M	A2
8a	M	A4	8c	M	A4
9a	M	A2	9c	M	A4
10a	M	A4	10c	M	A1

APPENDIX C

LITERATURE REVIEW

Discovering and Defining Stress

Historically, stress has been difficult to define due to its multidimensional nature. The term stress has been used to describe 1) stimuli that disrupt the physiological balance (i.e. homeostasis), 2) the state of an organism when homeostasis is challenged, and 3) the response to the homeostatic disruption (Levine, in press). A recent definition of stress, which takes into account the multiple aspects of stress, was provided by McEwen (2003). He defined stress as events that are deemed threatening to an organism (i.e. stressors) and result in both physiological and behavioral responses (i.e. stress response).

Walter Cannon (1871-1945) and Hans Selye (1907-1982) made some of the earliest contributions to the understanding of stress and were among the first to establish the involvement of the adrenal gland in the physiological response during various psychological states (Cannon, 1914) and to various stimuli (Selye, 1956). In a series of experiments, Cannon and colleagues observed increased discharge of adrenaline (i.e. epinephrine) from the adrenal medulla and increased mobilization of sugar in the blood with exposure to stimuli that invoked fear, rage, and pain. Borrowing McDougall's (1908) concept of fight or flight and Darwin's (1905) views concerning the survival of the organism, Cannon explained how the emotions of fear, rage, and pain would necessitate a physiological response that would arouse systems favoring survival. Selye's work focused on the role of the adrenal cortex hormone, glucocorticoid (GC), in the response and adaptation to a variety of stimuli. While conducting a study on ovarian tissue, Selye

made the incidental discovery that handling and daily injection of rats resulted in the development of peptic ulcers, enlarged adrenal glands, and immune tissue atrophy. He then discovered that chronic administration of multiple types of stressors (e.g. cold, heat, hemorrhage, and illness) resulted in similar physiological symptoms. Exposure to the stimuli also stimulated the release of GCs from the adrenal cortex. Since various stimuli resulted in similar physiological symptoms and responses, Selye proposed the General Adaptation model, which stated that the response to stress was non-specific and allowed the organism to adapt to various threatening events (Selye, 1956). Although we now know that stress responses can vary depending on the nature of the stressor (Levine, 2000; Singh, Petrides, Gold, Chrousos, & Deuster, 1999; Thrivikraman, Nemroff, & Plotsky, 2000), Cannon and Selye's findings had a profound impact on the field of stress physiology.

Physiological Stress Response Systems

The stress response is primarily mediated through interrelated neural and neuroendocrine systems. The sympathetic division of the autonomic nervous system is a rapid responder and regulates involuntary responses to threatening or arousing stimuli. When the sympathetic nervous system is triggered, postganglionic sympathetic neurons provide noradrenergic innervation to salivary, sweat and mucus-producing glands, the heart, skeletal muscles, lungs kidney, liver, stomach, pancreas, intestines, bladder, and reproductive organs. Innervation accelerates heart rate and blood pressure, relaxes airways, inhibits digestion, and stimulates glucose production and release (Bear, Connors, & Paradiso, 1996). Sympathetic postganglionic neurons also terminate in the adrenal medulla, stimulating the release of epinephrine into the blood stream, preparing the organism for the fight or flight reaction in the face of an immediate threat (Sapolsky, 2002).

The hypothalamic-pituitary-adrenal (HPA) axis is also activated in response to threatening stimuli. During HPA axis activation, stress-related inputs are ultimately relayed to the paraventricular nucleus of the hypothalamus (PVN), stimulating the release of corticotropin releasing factor (CRF) and arginine vasopressin. CRF and arginine vasopressin are released into the portal circulation of the median eminence and transported to the anterior pituitary where they stimulate the cleavage of proopiomelanocortin into adrenocorticotropic hormone (ACTH) (Cullinan, Herman, Helmreich, & Watson, 1995). Arginine vasopressin has a weaker effect on ACTH synthesis than CRF, possibly strengthening the effect of CRF on ACTH production and release rather than inducing ACTH production and release alone (Rivier & Vale, 1983). ACTH, a 39-amino acid peptide is released into systemic circulation and stimulates the production and release of glucocorticoids (GCs) from the adrenal cortex (Cullinan et al., 1995). CRF release stimulates heightened vigilance, defense-related learning and memory, and context-dependent motor responses such as freezing (Gunnar & Vasquez, 2006). GCs aid in the restoration of homeostasis through change, which McEwen termed allostasis. Allostasis allows the organism to adjust to short term environmental or psychological challenges that are necessary for survival (McEwen, 2003).

Hypothalamic-Pituitary-Adrenal Axis

CRF

CRF is a 41-chain peptide, believed to mediate endocrine, autonomic, and behavioral responses to stress (Vale, Spiess, Rivier, & Rivier, 1981). Although the highest density of CRF cell body concentrations are found in the medial parvocellular region of the hypothalamic PVN, CRF containing neurons are also found in extrahypothalamic areas such as the central nucleus of the amygdala, bed nucleus of the stria terminalis, hippocampus, nucleus accumbens, cerebellum,

lateral hypothalamus, and brain stem regions responsible for autonomic responses (Merchenthaler, Vigh, Petrusz, & Schally, 1982; Sawchenko et al., 1993; Swanson, Sawchenko, Rivier, & Vale, 1983). Thus, in addition to its role in the endocrine stress system, CRF operates as a neurotransmitter, mediating behavioral and autonomic responses to stress. CRF acts upon g-coupled protein receptors, activating adenylate cyclase. Three CRF receptors have been identified in the rodent brain: CRF1 (Chang, Pearse, O'Connell, & Rosenfeld, 1993; Perrin, Donaldson, Chen, Lewis, & Vale, 1993), CRF2a, and CRF2b (Lovenberg, Chalmers, Liu, & De Souza, 1995). The distribution of CRF 1 is primarily in the anterior pituitary, cerebellum, and cerebral cortex; the distribution of CRF2a is primarily in the septum, dorsal raphe nucleus, and ventromedial nucleus and the PVN of the hypothalamus; and the distribution of CRF2b is in the heart, skeletal muscle, cerebral blood vessels, and choroid plexus (Heinrichs & de Souza, 2001; Lovenberg et al., 1995). Two CRF receptors have been identified in primates: CRF1 and CRF2. CRF2 receptors are more widespread in primates, with both CRF1 and CRF2 receptors found in the cortex, pituitary, hippocampus, and amygdala of rhesus macaques (Sanchez, Young, Plotsky, & Insel, 1999). With regard to HPA activation, CRF 1 receptors seem to be responsible for activating the stress response by eliciting ACTH release from the anterior pituitary since CFR 1 receptors are highly abundant and CRF 2 receptors are barely detectible in the anterior pituitary. CRF interacts with CRF 1 receptors on corticotrophic cells of the anterior pituitary, stimulating the synthesis of proopiomelanocortin (POMC) molecule, which is the precursor to ACTH (Cullinan et al., 1995). Since CRF 2 receptors have a wider distribution in non-human primates, both receptor subtypes may play a role in stress-induced ACTH from the anterior pituitary (Sanchez et al., 1999).

ACTH and Glucocorticoids

ACTH is the primary releasing factor for GCs (cortisol in primates, corticosterone in rodents). ACTH acts upon adrenal cortex cell membrane receptors of the zona glomerulosa zone of the cortex and stimulates the rate limiting step of GC production, the conversion of cholesterol to pregnenolone. Pregnenolone is then converted to 17-a-OH-pregnenolone, then to 17-a-OH-progesterone, and to 11-deoxycortisol before the final production of GC (Michelson, Licinio, & Gold, 1995).

GCs act upon receptors located throughout the body, mediating allostasis or changes to maintain homeostasis. Since GCs have such widespread action, appropriate levels must be maintained (Walker Welberg, & Plotsky, 2002). The maintenance of GC basal levels and levels following stress activation is through a negative feedback mechanism, whereby GCs feedback to the level of the pituitary, hypothalamus, and higher brain regions (Cullinan et al., 1995). Two types of corticosteroid receptors are responsible for mediating the effects of GCs: type I or mineralocorticoid (MR) receptors and type II or glucocorticoid (GR) receptors. MR receptors are predominantly distributed in the hippocampus, amygdaloid nucleus, and in several cranial nuclei. GR receptors are distributed throughout the brain, but are heavily concentrated in the hippocampus, PVN, arcuate nucleus, locus coeruleus, and cortex. HPA hormone levels follow a circadian rhythm cycle, with an early morning peak and evening nadir. Superimposed on this cycle are the HPA hormone levels following stress (Reul, & de Kloet, 1985). MR receptors, which have a high affinity for GCs are primarily responsible for maintaining the circadian rhythm and basal levels (de Kloet & Reul, 1987). GR receptors have a low affinity and are generally occupied following stress or during the circadian rhythm peak, suggesting that stress-induced negative feedback is GR receptor mediated (Reul & de Kloet, 1985).

The effect of GCs on behavior and neuroendocrine activity depends on the type of receptor that is bound, the location of the receptor, and the ratio of MR to GR binding (de Kloet, 1991). Four different types of GC actions have been distinguished: permissive, suppressive, stimulating, and preparative. Permissive actions are typically associated with basal levels of GCs and are primarily mediated by MR receptors. Permissive actions precede any stressor and are important in maintaining basal levels so that the organism is capable of activating a stress response. Suppressive actions occur when there is a stress-induced GC release. Approximately one hour after the stressor, GC receptors respond to stress-induced GC increases by inhibiting the stress response to avoid deleterious effects of an ongoing stress response. GR receptors are primarily occupied at this time, which results in inhibition of systems that were activated during the stress response. Stimulatory actions that also occur after stress-induced release of GCs, have an onset of approximately 1 hour post stress. Like the permissive actions, the stimulating GC actions mediate the stress response. The stimulatory actions enhance the original stress response opposing the effects of the suppressive GC actions. MR receptors tend to facilitate the stimulatory actions while the GRs primarily mediate the inhibition of the ongoing responses brought about by increased GC levels. The opposing actions have been compared to turning on a fire hose to extinguish a fire (stimulatory actions) and shutting off or turning down the fire hose to decrease flood damage (suppressive actions). With these opposing actions, it is easy to see why high or low levels of GCs would have damaging consequences to the organism. For this reason the appropriate levels of GCs for proper functioning is in the shape of an inverted U. Unlike the other categories of GC actions, preparatory actions do not influence the immediate response to stress, but influence the way an organism will respond to future stressors. Increased GC levels can cause up-regulation of GR receptors, which can increase receptor sensitivity to

future stress-induced GC release. In sum the actions of GCs are important for regulating homeostasis through mediation of the stress response (Sapolsky, Romero, & Munck, 2000).

Stress Integration

Researchers often refer to the HPA axis as the limbic-hypothalamic-pituitary-adrenal axis to reflect the importance of limbic inputs in the mediation of the stress response. Among the limbic areas that have been implicated in HPA regulation are the hippocampus, prefrontal cortex, lateral septum, and amygdala (Brunson, Avishai-Eliner, Hatalski, & Baram, 2001; Feldman, Conforti, & Weidenfeld, 1995; Herman, et al., 2003; Herman, Tasker, Zeigler, & Cullinan, 2002; Lopez, Akil, & Watson, 1994; Vermetten & Bremner, 2002. Limbic mediation of the stress circuitry is dependent upon the characteristics of the stressor. Stressors can be categorized into 2 major types: systemic stressors and processive stressors. Systemic stressors are physical stressors (e.g. respiratory distress) that elicit a stress response, regardless of the context in which they occur. In contrast, processive stressors (e.g. novel environment) represent a possibly emerging threat and require psychological processing (Herman & Cullinan, 1997).

Evidence suggests that the stress circuitry varies between systemic type and processive type stressors (see Herman & Cullian, 1997 for review). Since systemic stressors may constitute an immediate threat to survival, they are relayed to the PVN by relatively direct pathways, bypassing cognitive processing. These pathways provide information concerning homeostatic disruption to the PVN that allows for a rapid response to the systemic stressors, mediated in part by the HPA axis (Herman & Cullinan, 1997; Herman et al., 2003). Processive stressors require the evaluation of a stressor as a potential threat (i.e. not an immediate challenge to homeostasis), which involves cognitive appraisal. The cognitive appraisal of a stressor's imminent threat is extremely important, given the energetic cost of mounting a stress response (Herman et al.,

2003). Limbic circuits are believed to mediate the cognitive and emotional aspects of processive stressors by integrating stress-related sensory information with information gained from previous experiences. Limbic areas are therefore able to increase or decrease HPA stress responsivity by channeling integrated information to the HPA axis (Herman & Cullinan, 1997).

Limbic Influences on the HPA axis

Hippocampus

One limbic area that has been implicated in the mediation of the HPA stress response is the hippocampus (Sapolsky et al., 2000). Evidence suggests that the role of the hippocampus on the HPA axis is inhibitory since lesions of the hippocampus result in increased plasma GC levels (Knigge, 1961). In addition to the role in regulating the HPA axis basal tone, the hippocampus is believed to mediate HPA negative feedback by acting as a brake to the axis following a stressor. Hippocampal lesions result in a prolonged HPA response, as measured by GC and/or ACTH release, to processive stressors such as restraint (Herman, Cullinan, Morano, Akil, & Watson, 1995) and acoustic stress (Nettles, Pesold, & Goldman, 2000), but have no effect on systemic stressors such as ether exposure (Herman, Doglas, & Carlson, 1998). Although there are no direct connections between the hippocampus and the HPA axis, evidence suggests that GABAergic interneurons of the bed nucleus of the stria terminalis relay information from the hippocampus to the PVN (Swanson, & Cowan, 1977).

Prefrontal Cortex

The prefrontal cortex (PFC) is also believed to provide inhibitory control of the HPA axis, but unlike the hippocampus is implicated only in HPA negative feedback, not HPA basal tone (Herman et al., 2003). ACTH and GC responses are enhanced following lesions to the anterior cingulate and medial PFC. These effects are demonstrated for processive stressors such

as restraint stress, but not for systemic stressors such as ether exposure. Application of GCs into the medial prefrontal region results in decreased ACTH and GC following restraint stress, but not following ether stress (Diorio, Viau, & Meaney, 1993). Although the evidence suggests a role of the PFC in the modulation of stress-induced negative feedback for processive stressors, there are no direct projections from the PFC to the PVN. However, pathways connecting areas of the PFC and areas, such as the bed nucleus of the stria terminalis that innervate the PVN do exist (Herman et al., 2002; van der Kooy, Koda, McGinty, Gerfen, & Bloom, 1984). It is believed that GABAergic interneurons project to the PVN, providing inhibition of the HPA axis (Sesack, Deutch, Roth, & Bunney, 1989).

Amygdala

The amygdala is another limbic structure that has been proposed as providing considerable modulation of the HPA axis, but unlike the hippocampus and the prefrontal cortex, it appears to activate rather than inhibit the HPA axis. Stimulation of the amygdala increases the production and release of GCs in rodents (Redgate & Fahringer, 1973) and non-human primates (Mason, 1959). The central nucleus of the amygdala (CeA) has been implicated in responses to processive stimuli; specifically those that provoke fear and anxiety (see Davis, 1992 for review). Lesions to the CeA result in reduced levels of ACTH and GCs following restraint stress (Beaulieu, DiPaolo, & Barden, 1986)). Monkeys with bilateral lesions of the CeA display reduced fearful behavior and have reduced levels of CRF when exposed to a snake and a threatening human (Kalin, Shelton, & Davidson, 2004) and lower levels of cortisol when exposed to a social stressor (Amaral, 2002). This evidence suggests the involvement of the CeA in HPA modulation of processive stressors associated with fear. Direct connections from the CeA to the PVN are limited (Gray, Carney, & Magnuson, 1989), but the CeA has connections

with structures, which directly innervate the PVN, such as the BNST (van der Kooy et al., 1984). Thus, the HPA axis may be activated through inhibitory output of the CeA and inhibitory output from the bed nucleus of the stria terminalis, creating an excitatory net effect (Herman, 2003).

HPA axis and Early Modification

The HPA axis is highly susceptible to modification early in development (Matthews, 2002). It is believed that one of the most salient features of a social organism's early environment is the presence of the primary caregiver or attachment figure. The infant's sensory system presumably monitors information from the environment, such as the mother's location and accessibility. The sensory information and learned information regarding the attachment figure's usual response to the infant's behavior is integrated into internal models of the physical environment, the social environment, the attachment figure, and the self. It is believed that these models are important determinants in how the infant appraises the environment and how it achieves its goals. According to attachment theory, if the infant develops a secure attachment with its primary caregiver, the infant may explore its environment and return to the mother when stressful stimuli are encountered. In this way, the attachment relationship would be able to support physiological homeostasis by providing contact comfort to the infant when exposed to stressors. The caregiver's response to the infant is believed to provide the mechanism that enables the infant to effectively cope (emotionally and physiologically) to stressors in the environment. The mother-infant interactions can also lead to long-term changes in stress responsivity and emotional regulation (Cirulli, Berry & Alleva, 2003; Kraemer, 1992). Since attachment theory presumes that certain mother-infant interactions are necessary for normal development, it has been hypothesized that disruption of the mother-infant relationships may lead to abnormal behavior and physiology. Rodent and non-human primate studies that disrupt

the mother-infant relationship have been used to examine the consequences of mother-infant relationship disruption.

Effects of Maternal Infant Interactions on HPA regulation

Evidence from Rodent Models

The rodent model is often used to characterize the effects of maternal separation on behavior and physiology. The developmental period of the rat pup is relatively short and the physiology of the HPA axis undergoes rapid developmental changes within the first two weeks of life (Hennessy, 1996). The period between post-natal day (PND) 4 and PND 14 in the rodent has been termed the stress hyporesponsive period. During this period pups are non-responsive to most stressors and have low basal corticosterone (CORT) and ACTH levels, which may act to shield the brain from excess exposure to glucocorticoids (Walker, Perrin, Vale, & Rivier, 1986). Although pups do not mount a stress response to most stressors during this period, they will respond to these stressors if maternally separated for more than one hour. Pups separated at PND 12 for 8 and 24 hours and exposed to a novel environment had higher CORT levels than baseline. Separations for 0 and 1 hour did not elevate CORT levels over baseline when exposed to the novelty stressor. Since prolonged periods of maternal separation were necessary to enhance the pup's response to stressors, it was hypothesized that the mother was providing a regulatory influence on the pup (Stanton, Gutierrez, & Levine, 1988).

Because longer separations seem to be necessary to impact HPA axis regulation, single 24 hour maternal separations have become a standard paradigm to investigate the effects of maternal separation on HPA axis regulation. Through the use of this paradigm, it has been discovered that the effects of a single 24 hour separation depend on the time period in which the separation occurs. Rodents deprived of maternal contact early in life (PND 3) hyper-secreted

ACTH while those deprived late (PND 11) hypo-secreted ACTH when exposed to a mild stressor just prior to weaning (PND 20). There were no significant differences between the non-deprived, early, or late deprived groups for stress-induced cortisol levels or basal levels of ACTH and CORT. Interestingly, changes in ACTH responsivity were accompanied by changes in CRF gene expression in the PVN. Early deprived animals with exaggerated ACTH responses also had elevated stress-induced CRF mRNA in the PVN compared to controls. Late deprived animals with attenuated ACTH responses had reduced stress-induced CRF mRNA in the PVN. This study demonstrated that rodents are able to mount a HPA response to stress during the stress hyporesponsive period when they are exposed to a single 24 maternal separation and that the persistent effects are different depending on the developmental time period in which the insult occurred (van Oers, de Kloet, & Levine, 1998).

There is evidence that suggests that CORT elevations during early development lead to suppression of HPA activity later in life (Erkskine, Geller, & Yuwiler, 1979). Therefore, it was hypothesized that the attenuated ACTH response of the late deprived pups was due to separation-induced chronic CORT secretion. This idea is supported by the fact that the late deprived pups, but not the early deprived pups, demonstrated an increase in CORT secretion during the separation (van Oers, de Kloet, & Levine, 1999). In order to test this hypothesis, dexamethasone (synthetic glucocorticoid) injections (treated) or saline injections (non-treated) were given to deprived or non-deprived pups at PND 11. The dexamethasone injection was given prior to deprivation to prevent CORT elevations during the period of separation. Although dexamethasone injections were successful at preventing the separation-induced rise in CORT, dexamethasone did not prevent all of the deprivation induced changes seen later in development.

Therefore it was believed that CORT elevations during separation are not solely responsible for persistent changes in HPA axis regulation (Van Oers, 1998; 1999).

According to Hofer (1983) there are specific forms of maternal stimuli that are responsible for regulating the rodent's HPA axis early in development. It was hypothesized that it was the absence of these specific maternal cues, not only separation-induced CORT elevations that were responsible for the persistent changes seen in rodents exposed to maternal separation. To determine the effect of stimuli associated with maternal behavior on physiological regulation, single, 24 hour maternal separation paradigms have been developed where researchers mimic maternal behavior, such as stroking and/or feeding, during the period of separation. The results demonstrated that feeding did influence HPA basal tone and responsivity. Deprived pups that were fed and stroked had lower basal and stress induced CORT than deprived pups that were only stroked. Although there were no differences between the groups for ACTH levels (basal or stress-induced), the results suggest that feeding may act to desensitize the adrenal to ACTH, resulting in lower CORT levels (Suchecki, Rosenfeld, & Levine, 1993). A similar study also found that maternally deprived fed and stroked pups had similar ACTH and CORT responses compared to non-deprived pups. However deprived pups that were stroked only had similar ACTH responses to non-deprived pups, but resembled deprived and non-stroked/non-fed pups with regard to stress induced CORT levels. Additionally, deprived pups that were fed and stroked also showed similar CRF expression in the PVN to non-deprived pups (Suchecki et al., 1993; van Oers de Kloet, Whelan & Levine, 1998; van Oers, de Kloet, & Levine, 1999). These studies suggest that changes induced by maternal separation are a result of the absence of feeding and stroking rather than solely due to increased CORT during the separation period. The studies also demonstrate that different aspects of maternal stimuli may mediate different aspects of

neuroendocrine activity. These findings are consistent with the view that specific maternal stimuli are capable of regulating physiological systems during early development.

Repeated separation paradigms have also been used to discover the consequences of maternal separation. Many rodent repeated separation paradigms consist of handling and maternal separations for 15 minutes (HMS15) or 180 minutes (HMS180), with control groups usually consisting of dam-reared, non-handled pups or animal facility reared animals that are dam-reared with minimal handling for routine cage cleaning. These paradigms have demonstrated that even short periods of repeated separation can induce behavioral and HPA axis alterations (Huot, Gonzalez, Ladd, Thrivikraman, & Plotsky, 2004; Huot, Thrivikraman, Meaney, & Plotsky, 2001; Ladd, Huot, Thrivikraman, Nemeroff, & Plotsky, 2004). HMS180 rats have been shown to exhibit prolonged ACTH secretion when exposed to stressors such as air puff startle (Ladd et al., 2004; Huot et al., 2004; 2001) and restraint stress (Liu, 2000) in adulthood. ACTH levels of the HMS180 group peaked at 5 minutes and 30 minutes post air puff startle (APS) and returned to baseline at 45 minutes post APS. HMS15 rats had a single ACTH peak at 5 minutes post APS and returned to baseline at 10 minutes post APS (Ladd et al., 2004). HMS180 rats also had greater ACTH secretion in response to the APS than HMS15 rats (Huot et al., 2001). An additional study showed that HMS180 rats had greater and more prolonged ACTH levels than both HMS15 and non-handled rats (Huot et al., 2004). However, another study showed that non-handled rats are similar to HMS180 rats compared to HMS15 rats, depending on the time of measurement. Restraint stress produced enhanced ACTH secretion in HMS180 and non-handled rats compared to HMS15 rats during and shortly after the stressor; however ACTH levels in the HMS180 rats were higher than both the non-handled and the

HMS15 at 100' following the termination of restraint (Kalinichev, Easterling, Plotsky, & Holtzman, 2002).

CORT responses followed a similar pattern. HMS180 CORT levels peaked at 45' and HMS15 CORT levels peaked at 10' post APS (Ladd, 2004). HMS180 rats also had higher CORT at 45' post APS (Huot, 2004) and higher integrated CORT levels (Huot, 2001) following the APS stressor. A brief handling stressor resulted in higher CORT levels in HMS180 males than HMS15 and non-handled males. However, no differences were found between female HMS180, HMS15, and non-handled rats, suggesting gender differences in the long term effects of maternal separation (Kalinichev et al., 2002).

Further evidence of HPA alterations with the repeated separation paradigm come from studies that utilize the dexamethasone (DEX) suppression test. DEX is injected and stress hormone levels are measured several hours following the injection. Several hours following an injection of DEX, stress hormone levels should be low, indicating appropriate negative feedback. Although levels of ACTH were low for both the HMS15 and HMS180 rats 6 hours following the DEX injection, levels were elevated in the HMS180 rats compared to the HMS15 rats 7 and 8 hours following the injection. Seven to eight hours post injection coincided with the normal 7-8 pm diurnal rise in ACTH levels. The DEX did not suppress the HMS180's diurnal rise in ACTH, which suggests that HMS180 rats had an early escape from the negative feedback (Ladd et al., 2004). This early escape is indicative of impaired negative feedback.

Physiological changes in response to repeated maternal separation are also reflected in brain regions associated with the stress response system. HMS180 rats had higher CRF gene expression in the PVN than HMS15 (Huot, 2004) and both HMS15 and animal facility reared control rats (Plotsky & Meaney, 1993). Glucocorticoid receptor gene expression in the cingulate

gyrus and frontal cortex were lower in the HMS180 rats than in the HMS15 rats (Ladd et al., 2004). CRF secretion may drive the enhanced ACTH secretion seen in the HMS180 rats. Decreased glucocorticoid receptor gene expression may decrease negative feedback on the axis, prolonging the ACTH and CORT response to stressors as seen in the HMS180 rats.

There is some evidence that the long term alterations in the stress response system caused by repeated 3 hour maternal separations can be prevented (Huot, 2001; Huot, 2004). Rats treated with a selective serotonin reuptake inhibitor class of antidepressant had decreased ACTH and CORT stress levels compared to non-treated and treated HMS15 rats. When rats were given access to ethanol HMS180 rats consumed more alcohol than the treated HMS180 and HMS15 rats, HMS15 rats, and animal facility reared rats. The antidepressant had no effect on the ethanol consumption of the animal facility reared or HMS15 rats. Furthermore, ethanol consumption was positively correlated with CORT response to stressors and negatively correlated with time spent in open arms during the open arm maze task. This suggests that the repeated separation paradigm may result in enhanced anxiety-like behavior in addition to alterations in the stress response system (Huot et al., 2001).

Non-pharmacological treatments have also been successful in reversing the effects of repeated maternal separation. When dams of HMS180 pups were placed with a foster litter during separation from their own litter, the pups (HMS180F) resembled HMS15 pups rather than HMS180 pups. HMS180F secreted less ACTH and CORT and returned to baseline faster after APS than HMS180 rats. There were no significant differences in CORT or ACTH levels between the HMS180F rats and the HMS15 rats. Foster litters also partially normalized CRF levels in the PVN since the HMS180F rats had CRF levels that were between the levels of the HMS15 and HMS180 rats (Huot et al., 2004).

Rodent animal models have demonstrated that early maternal separation can influence HPA axis regulation and behavior both immediately and persistently. These models have also shown that the specific outcomes are determined by the timing of the maternal separation (early (PND3) vs. late (PND11)) (van Oers, de Kloet, Whelan, & Levine, 1998), the length of the separation (Stanton et al., 1988), the maternal behavior upon reunion with the dam (Huot, 2004), and "maternal-like" stimuli that are given during the separation period (van Oers, de Kloet, & Levine, 1998; van Oers et al., 1999).

Evidence from Primate Models

In most species of primates, the infant remains in constant contact with the mother for the first few weeks of life. Separation of the infant from the mother in primates is regarded as an extremely stressful procedure (Harlow & Harlow, 1962; Kaufman, 1973). This assumption is based on observations of infants following separation from their mothers. Infant behavior following separation has been characterized by two distinct phases: 1) the protest phase during which infant vocalization and locomotor activity increase and 2) the despair stage where infants become inactive and remain in a slouched or huddled position (Hinde, Spencer-Booth, & Bruce, 1966; Kaufman & Rosenblum, 1967). Both phases are believed to be adaptive responses resulting from disruption of the mother-infant attachment relationship. The purpose of the protest behavior is to regain contact with the attachment figure (Kraemer, 1988) since this behavior normally results in the mother responding to the infant's needs and providing emotional and physical security (Kaufman & Rosenblum, 1967; Levine, 1987). If contact is not regained by the protest behavior, it is believed that suppression of the costly behavior occurs. According to Kaufman & Rosenblum (1967) the despair behavior is also adaptive since the infant can conserve energy until it is rediscovered by the mother. Humans also demonstrate the bi-phasic

response during separation (Bowlby, 1960), which suggests similarities in attachment bonds between human and non-human primates as well as similarities in behavioral and biological systems responsible for responding to attachment disruption (Kraemer, 1988). These proposed similarities form the basis for utilizing primate maternal separation paradigms as models for human infant isolation and neglect.

Early studies utilizing maternal separation paradigms were designed to develop a non-human primate model of human social isolation in order to gain insight into the deleterious effects of isolation on cognitive, social, and physical development. Harlow and colleagues (1965) found that effects of total isolation (no auditory, visual or tactile contact with animals or humans) for the first 3, 6, or 12 months of life had deleterious effects that depended on the age and duration of isolation. Monkeys isolated at 3 months showed the typical phase of agitation and despair during the separation, but did not show immediate cognitive impairment or long-term social behavior impairment. Harlow equated these effects to those seen in children that are quickly rescued from socially isolated environments. Monkeys reared in isolation for the first 6 months or first year of life did show abnormalities in post-isolation social behavior. Six-month isolates showed a decrease in social play compared to 3-month isolates and controls. Twelve-month isolation resulted in even more devastating effects (Harlow, Dodsworth, & Harlow, 1965). Social behavior was “almost obliterated” as demonstrated by abnormally low levels of social play, social approach, and social threat (Harlow et al., 1965, pg. 94). No cognitive effects were seen in the animals isolated at either 6 or 12 months. Later isolation (isolation for 6 months starting at 6 months or one year month) did influence fearful and aggressive behavior, with individual differences ranging from complete fearlessness to excessive fear and lack of aggression to extreme aggression. Interestingly, for some individuals their extreme fearful and

aggressive behavior was context dependent, showing extreme fear under some circumstances and fearlessness in others (Harlow et al., 1965).

As previously discussed, the HPA axis mediates the response to stressors. Since maternal separation is regarded as a highly stressful procedure, transient and long-term HPA alterations were regarded as potential consequences of maternal separation. The assumption of long-term alterations was based on the theoretical rationale that early maternal stimulation sets the level of HPA axis regulation. Transient HPA alterations have been demonstrated through different rearing paradigms. Infants removed from their mothers and reared in a nursery by researchers (i.e. nursery-reared) demonstrated higher basal cortisol at PND 14 and 30 than mother-reared infants (Champoux, Coe, Schanberg, Kuhn, & Suomi, 1989). When rhesus macaques were separated from their mothers and reared with peers, they had higher basal ACTH levels at month 1 compared to mother-reared monkeys although basal cortisol levels were equal at month 1 (Clarke, 1993).

Long-term HPA alterations have also been detected in maternally separated monkeys. Peer-reared rhesus macaques had lower basal cortisol at PND 60 than mother-reared macaques. However, cortisol levels of peer-reared and mother-reared monkeys did not differ when exposed to 30 minute maternal separations at PND 90, 120, or 150, which suggested that HPA alterations resulting from rearing were not long-lasting (Shannon, Champoux & Suomi, 1998). In contrast, Clarke (1993) did find long-lasting HPA differences between mother-reared and peer-reared monkeys. At 6 months, peer-reared monkeys had a lower ACTH response to stressors than mother-reared monkeys. Although basal levels of cortisol were equal for the two rearing groups, the peer-reared monkeys showed a smaller cortisol response to the mild stress. cortisol responses to the severe stress were equivalent, possibly due to a ceiling effect (Clarke, 1993). These long-

term effects suggest suppression of the HPA axis due to early HPA hyper-activity caused by early maternal separation as previously described in rodent models (van Oers et al., 1999).

Studies have also found opposite HPA effects resulting from peer-rearing (Fahlke et al., 2000; Higley, Suomi, & Linnoila, 1991; Meyer & Bowman, 1972; Sackett, Bowman, Meyer, Tripp, & Grady, 1973). Higley and colleagues (1991) found that peer-reared rhesus macaques had higher basal and stress-induced (social separation) cortisol levels than the mother-reared macaques, which were consistent across the first two years of life. A similar study also showed that peer-reared rhesus macaques had greater stress-induced cortisol levels than mother-reared infants when exposed to social separation at 6 months of age (Fahlke et al., 2000). A study comparing nursery-reared and peer-reared monkeys provided similar evidence of HPA enhancement as a result of maternal separation. Nursery-reared rhesus macaques had higher basal cortisol than those that were peer-reared. Cortisol responses to ACTH injection and to exposure to a novel room did not differ between the two rearing groups. Behaviorally, nursery-reared monkeys showed greater fear behavior, such as self-clutching, rocking, and withdrawal, but surprisingly rates did not correlate with basal cortisol levels. Socially, nursery-reared animals had less physical contact with peers when introduced to a social group than peer-reared monkeys, suggesting long-term social deficits (Sackett et al., 1973).

Meyer & Bowman (1972) investigated HPA alterations resulting from maternal separation into adulthood. Rhesus macaques that were reared during the first 9 months under total isolation, partial isolation (visual and auditory exposure to other isolates), or reared in the wild for the first 12-24 months of life were tested later in life. Isolation reared monkeys were characterized as emotional and hyper-reactive at 4-5 years of age. However, HPA alterations were not detectable at 3-4 years post-treatment. There were no differences in the cortisol

responses to mild stress (saline injection), severe stress (2 hours in a restraint chair) or in response to ACTH injections between the feral, total isolates, or partial isolates. Surprisingly the long-term behavioral consequences of isolation were not accompanied by exaggerated cortisol response to stress or ACTH administration (Meyer & Bowman, 1972).

The studies do seem to indicate that infants reared without mothers have higher basal levels of cortisol or ACTH, suggesting a more active HPA axis (Champoux et al., 1989; Clarke, 1993; Higley et al. 1991). However, long-term effects are mixed. Studies have found no long-term effects (Meyer & Bowman; Shannon et al., 1998), hypo-responsiveness to stressors (Clark, 1993), enhanced basal tone (Higley et al. 1991 ; (Sackett et al., 1973), and hyper-responsiveness to stressors (Fahlke,et al., 2000; Higley et al., 1991; Sackett et al., 1973)) in maternally separated rhesus macaques.

The isolation paradigms demonstrated transient and long-term behavioral and HPA alterations. One of the major flaws in the isolation studies is that infants also experience isolation from members of their species. Although peer-rearing studies attempted to remedy this problem, a more naturalistic approach has been taken, utilizing repeated maternal separations or brief single separations. Another major problem comes into play when trying to compare results across studies. Variations in isolation procedures are extreme and make it nearly impossible to interpret the maternal deprivation data as a whole (Chappell & Meier, 1975). Using a repeated or single brief separation model solves these problems by allowing for comparisons before and after the manipulation. It also keeps control groups and experimental groups similar, other than the brief separations, making interpretation less difficult.

Dettling, Feldon & Price (2002) conducted a repeated maternal separation paradigm similar to those used with rodents in non-human primates. Common marmosets (*Callithrix*

jacchus) were removed from their social groups daily between 30 and 120 minutes from PND 2 to 28. At PND 28, the maternally separated marmosets weighed less and had lower morning basal cortisol than the controls (non-maternally and non-handled). Social behavior upon reunion with the mothers also varied between the experimental and control groups. Maternally separated marmosets spent more time in the suckling position, spent more time vocalizing, and less time in social play than controls. However, unlike in the rodent studies, maternal behavior did not differ between the experimental and control mothers. Rodent studies suggested that maternal behavior upon reunion influenced the physiological effects of maternal separation (Huot et al., 2004). Since maternal behavior was not affected, the HPA effects were more likely a direct result of the separation procedure rather than changes in maternal behavior toward the infant upon reunion. It should be noted that some of the mothers were left with another infant when separated from their infants. In the rodent literature, providing a dam with a foster litter prevented changes in maternal behavior upon reunion with the pups and this also prevented HPA alterations in the pups (Huot et al., 2004). This study showed HPA alterations even though there were no apparent changes in maternal behavior (Dettling et al., 2002).

Evidence from human studies

Evidence that early experience is critically involved in normative physiological and behavioral development comes from studies investigating the outcomes of children exposed to various adverse conditions or stressors. Although adverse conditions can include disasters, such as earthquakes or tsunamis or witnessing traumatic events, such as a car accident, this review will focus on adverse experiences involving rearing experiences and parental care, such as child maltreatment. For normative development a caregiving environment needs to provide expectable experiences in a nurturing and sensitive manner (Cicchetti & Lynch, 1995). Since the parents are

the most salient features of the child's environment in early development, disruptions in the parent-child relationship can have devastating effects on both psychological and biological processes and increase the vulnerability to psychopathology later in life (Cirulli, Berry, & Alleva, 2003).

One approach into the investigation of disruptions in the early caregiving environment has been to study children of depressed mothers. Children of depressed mothers are likely to face disruption of the mother-infant relationship. Depressed mothers express less positive and more negative affect when interacting with their infants and are less responsive to the emotional needs of the infant. These behaviors are possibly stressors for the infant since the mother is not able to buffer the infants stress response in a predictable way (Ashman, Dawson, Panagiotides, Yamada, & Wilkinson, 2002). To determine the effects of the maternal style of the depressed mothers, Ashman and colleagues (2002) measured the cortisol responses of 7-8 year old children of chronically depressed, early depressed mothers (depressive episodes during the first 1-2 years of the child's life) and non-depressed mothers. Although no differences were found between the groups for cortisol, there were differences with regard to clinical symptomology. Children of chronically depressed mothers were more likely to have clinical levels of externalizing behavior (26.8%) than children of non-depressed mothers (0%) and early depressed mothers (6.7%). Children of depressed mothers were not more likely to have clinical levels of internalizing behavior. A similar study found that children of depressed mothers demonstrated higher levels of aggression toward their mothers. The infants of depressed mothers had reduced generalized frontal electrical brain activity, which was negatively correlated with the mother's depression severity and with the infant's levels of aggression. (Dawson et al., 1999). Genetic factors may have played a role in the behavioral and physiological outcomes but, the early depressed control

group does help control for the confound of genetic factors. Although genetic factors can not be ruled out, it is possible that the children of depressed mothers failed to develop adequate emotional regulation as a result of inappropriate maternal care.

The study of children reared in deprived conditions, such as poorly run orphanages, has provided further evidence of the effects of early social and emotional deprivation. In one study, institution-reared children had lower than normal morning cortisol levels and failed to demonstrate the normal afternoon decrease in cortisol (Carlson, Dragomir, Earls, Farrell, Macovei, Nystrom & Sparling, 1995; Carlson & Earls, 1997).

Studies of children exposed to childhood maltreatment, such as physical, sexual, and emotional abuse have also been used to discover the behavioral and physiological effects of early adverse experiences. There is substantial evidence that disrupting the normal infant-caregiver relationship in humans impacts neurobiological stress-response systems, including the HPA axis (De Bellis, 2000; De Bellis et al., 1994; De Bellis et al., 1999; Heim et al., 2000; Heim & Nemroff, 1999; Pine, 2003; Yehuda, Halligan, & Grossman, 2001; Cicchetti & Rogosch, 2001a; 2001b). Changes in these neurobiological systems are associated with changes in behavioral and emotional regulation (Ashman, Dawson, Panagiotides, Yamada, & Wilkinson, 2002; Flinn & England, 1995; Hardie, Moss, Vanyukov, Yao, & Kirillovac, 2002; Pine, 2003 and an increased vulnerability to mental illness (De Bellis et al., 1999; Heim, Ehlert, & Hellhammer, 2000; Heim & Nemroff, 1999; Raison & Miller, 2003). According to Cicchetti and Lynch (1995), child maltreatment (i. e. physical, emotional, or sexual abuse or neglect) represents a profound failure of the caregiving environment to provide expectable experiences that are necessary in order to facilitate normal development. This failure of appropriate caregiving is believed to be stressful and traumatic and may produce biological and behavioral consequences because of the exposure

to chronic stress during development. For this reason, a large number of studies have focused on the association between HPA activity and childhood maltreatment (see Bremner & Vermetten, 2001; De Bellis, 1999; Gunnar & Vazquez, 2001; Raison & Miller, 2003; Vermetten & Bremner, 2002 for review). A study of maltreated children (74.3% emotionally abused, 79.6% neglected, 37.1% physically abused, and 17.4% sexually abused) found that physically abused children had a tendency for lower morning cortisol and demonstrated a flattened diurnal decrease in cortisol compared to non-maltreated children. Additionally 31.4% of the physically abused children had extreme low cortisol levels. Children that were sexually and physically abused had higher rather than lower AM cortisol levels compared to emotionally abused, physically abused, and neglected children (Cicchetti & Rogosch, 2001).

Child maltreatment is associated with a host of psychiatric disorders, such as depression, anxiety disorders, and conduct disorders (Cicchetti & Rogosch, 2001b; Cohen, Brown, & Smailes, 2001; Yehuda et al., 2001), and a variety of stress response patterns (Cicchetti, 1995; Cicchetti & Rogosch, 2001a; 2001b; De Bellis et al., 1994; Gunnar & Vazquez, 2001; Heim et al., 2000; Yehuda et al., 2001). Changes in HPA regulation usually accompany the behavioral outcomes, but the particular characteristics of the HPA dysregulation seem to depend on psychiatric status. Below is a review of studies that have investigated HPA axis activity of maltreated individuals that have been diagnosed with depression, anxiety, or conduct disorders.

Depression

HPA dysregulation is commonly seen in adults suffering from depression (see Pariante, 2003 for review) and is also seen in depressed individuals that have undergone maltreatment as children (De Bellis, 1994; Heim, 2002; Heim & Nemroff, 2000; Kaufman, 1997). Kaufmann (1991) found that individuals maltreated as children and diagnosed with depression showed a

rise in cortisol over the day, rather than the normal decline (Kaufmann, 1991). However, a study of 7-15 year old sexually abused girls showed normal 24 hour urinary free cortisol levels and lower basal levels of ACTH compared to controls (De Bellis et al., 1994). Conflicting results with regards to basal HPA function are resolved when abuse status is considered. Yehuda and colleagues (2001) found that children with depression or post-traumatic stress disorder (PTSD) that suffered an emotional type of abuse had low mean cortisol values, while children with depression or PTSD that suffered physical or sexual abuse had high cortisol.

HPA axis differences in maltreated individuals with depression have also been discovered through the use of psychological and pharmacological challenges. CRF administration and exposure to stressors should result in increased cortisol and ACTH responses. Sexually abused girls demonstrate a blunted ACTH response, but normal cortisol response to the CRF challenge. However, the finding of blunted ACTH to CRF seems to only hold true for those children that are no longer in the maltreatment environment. Children in situations of ongoing maltreatment and diagnosed with depression showed an increased rather than a decreased ACTH response to CRF administration (Kaufman et al., 1997). Women with a history of childhood abuse and clinical depression showed higher ACTH and cortisol responses to a psychological stressor (Heim, Newport, Bonsall, Miller, & Nemroff, 2001) and a lower ACTH response to CRF administration when compared to controls (Heim et al., 2000).

Anxiety Disorders

Anxiety may represent an exaggerated anticipatory response to stress. Anticipatory responses include freezing, sympathetic nervous system activation, HPA axis activation, and heightened reflexive responses, such as startle responses (Rosen & Shulkin, 1998). Therefore, it is not surprising that HPA alterations are associated with pathological anxiety (De Bellis, 1999

part I). Numerous studies have found elevated basal cortisol levels in children suffering from anxiety disorders (see Gunnar & Vasquez, 2006 for review). Childhood maltreatment is a major risk factor for the development of anxiety disorders, including PTSD (see Pine & Cohen, 2002 for review). Children neglected during infancy demonstrating anxious or withdrawal behavior have been found to have high salivary cortisol levels, while maltreated children demonstrating antisocial behavior had low cortisol levels (Flinn & England, 1995). Pre-pubertal children with PTSD with a history of maltreatment also demonstrated higher cortisol levels than controls, and cortisol levels were positively associated with the duration of the PTSD trauma and the severity of the PTSD symptoms (De Bellis et al., 1999). However, women sexually abused as children, with and without PTSD, show prolonged cortisol suppression to dexamethasone compared to controls (Stein, Yehuda, Koverola, & Hanna, 1987). This finding in adults is logical since adults suffering from trauma-induced PTSD frequently demonstrated low basal cortisol levels and prolonged cortisol suppression to dexamethasone (see Yehuda, 2000). Together the data suggests that there may be a transition from hyper- to hypo-functioning at the level of the adrenal when transitioning from childhood to adulthood as a result of prolonged adversity (Yehuda et al., 2001).

Disruptive Behavior Disorders &Externalizing Problems

Disruptive behavior disorder includes conduct disorder and oppositional defiant disorder (ODD), which are both characterized as persistent disruptive, antisocial, and aggressive behavior (i.e. externalizing problems). Most models of disruptive behavior disorders presume that children at risk for the development of the disorder are under-reactive to threatening stimuli and therefore do not appropriately respond to the stimuli. It is also believed that these at-risk children suffer from under-arousal and may use sensation seeking to compensate for the under-arousal (Burke,

Loeber, & Birmaher, 2002). Since the HPA axis is important mediator in responding to stressful and threatening stimuli, studies have focused on the association between disruptive behavior disorder and HPA axis regulation (Pajer, Gardner, Rubin, Perel, & Neal, 2001; Shoal, Giancola, & Kirillova, 2003; van Goozen et al., 1998).

Disruptive behavior disorder and clinical levels of externalizing problems are associated with low basal levels of cortisol (Cicchetti & Rogosch, 2001; Hart, 1995; Pajer et al., 2001; Shoal et al., 2003; van Goozen et al., 1998). Boys diagnosed with Oppositional Defiant Disorder (ODD) were found to have lower salivary cortisol than control boys. Although cortisol responses to stress did not differ between the two groups, when the ODD group was divided into either highly anxious or low in anxiousness, differences were discovered. ODD boys with high levels of anxiety had higher cortisol responses to stress, while ODD boys with low anxiety had lower cortisol stress responses (van Goozen et al., 1998). Another study found that boys with persistent aggression problems and early onset of aggression had low salivary cortisol levels (McBurnett, Lahey, Rathouz, & Loeber, 2000). Similar associations have been found in females. Adolescent girls with conduct disorder also showed lower plasma cortisol levels throughout the day (Pajer et al., 2001). Low cortisol levels have also been shown to predict future problem behavior. Pre-adolescent boys that had low cortisol levels were more aggressive during middle adolescence. Low self control was found to be the most important mediator of low cortisol levels and later aggressive behavior (Shoal et al., 2003).

Aggressive and antisocial behavior (Cohen, Brown, & Smailes, 2001; Dodge, Lochman, Harnish, Bates, & Pettit, 1997; Dodge, Pettit & Bates, 1997), disruptive behavior disorder (Becker & McCloskey, 2002; Ferguson, Horwood, & Linskey, 1996; Flisher et al., 1997) and general externalizing problems (Cicchetti & Rogosch, 2001; Hart Gunner, & Cicchetti, 1995;

Hart Gunner, & Cicchetti, 1996; Keiley, Howe, Dodge, Bates, & Petit, 2001; Thornberry, Ireland, & Smith, 2001) are well-established outcomes of child maltreatment. The HPA activity of maltreated individuals with disruptive behavior disorder or externalizing problems tends to be hypo-cortisolomic. Maltreated children assessed as more behaviorally reactive had lower morning basal cortisol levels (Hart et al., 1995). A subsequent study by Hart and colleagues (1996) found that maltreated children with clinical externalizing symptoms also had lower cortisol levels than maltreated children without externalizing symptoms (Hart et al., 199). In a study by Cicchetti and Rogosch (2001), maltreated children were more likely to have externalizing problems but equally as likely to have internalizing problems as non-maltreated children. Individuals with externalizing problems had lower morning basal cortisol levels than those with internalizing problems. For girls only, maltreatment accompanied by externalizing problems resulted in lower cortisol than maltreatment alone (Cicchetti & Rogosch, 2001). The data suggest that disruptive and aggressive behavior in maltreated children is associated with lower basal levels of cortisol, which supports the idea that these children are under-aroused and use risky and inappropriate behavior to compensate for the under-arousal.

Relationship Disruption and Growth

In addition to HPA functioning and behavioral alterations, early adverse experience can also impact growth processes. In humans, psychosocial dwarfism or abuse dwarfism and failure to thrive are documented conditions associated with infant maltreatment (Kerr & Black, 2000; Money, Annecillo, & Kelley, 1983; Olivian, 2003). Psychosocial dwarfism is characterized by lower than normal height and body weight, delayed onset of puberty, and delayed cognitive and social development (Johnson, Kamilaris, Chrousos, & Gold, 1992). Failure to thrive diagnosis is based solely on retarded growth rate (Kerr & Black., 2000). Despite reported cases of

psychosocial dwarfism and failure to thrive in maltreated children, few researchers have focused on the effects of maltreatment on growth processes. It is unclear why this physiological consequence has been overlooked in the field, especially since glucocorticoids, which are often studied in relation to infant maltreatment, are known to influence the growth hormone system. Growth hormone (GH) secretion is controlled by both growth hormone-releasing hormone and (GHRH) and somatostatin. Secretion of GH from the pituitary is stimulated by the release of GHRH from the arcuate nucleus. Inhibition of GH and GHRH release is through somatostatin, released from the parventricular nucleus. GH also has a negative feedback effect on this neuroendocrine axis by stimulating the release of somatostatin, which eventually results in the attenuation of GH release (Holboer, 1995). The activity of the growth hormone system is influenced under times of stress through the activity of the HPA axis. During chronic stress, glucocorticoids inhibit the release of GH and GHRH as well as decrease sensitivity of tissue to the effects of GH and additional growth factors stimulated by GH (Johnson et al., 1992. Evidence also suggests that HPA activation decreases GH production by increasing somatostatin (Charmandari, Kino, Souvatzoglou, & Chrousos, 2003; Wehrenberg, Janowski, Piering, Culler, & Jones, 1990).

Growth failure in children living in stressful environments has been demonstrated in several studies (Albanese et al., 1994; Charmandari et al., 2003; Ellis, Fisher, & Zaharie, 2004; Pears & Fisher, 2005; Skuse, Albanese, Stanhope, Gilmour, & Voss, 1996). Preschool-aged foster children were shorter for their age than non-maltreated control children. This finding may have been due to the maltreatment history of the foster children since a negative association between height for age and experiences of neglect was found (Pears & Fisher, 2005). In a sample of children reared in a Romanian orphanage, anxiety symptoms and disruptive behaviors were

associated with delayed physical growth. Smaller stature was associated with increased anxiety symptoms, while relatively larger stature was related to increased disruptive behavior and decreased anxiety symptoms. Although there were no detectable associations between delayed growth and duration of institutionalization, it is important to note that even the larger stature children were 1.6 standard deviations below the mean stature for age (Ellis et al., 2004). This suggests that even within the realm of physical growth delays, physical size may be related to psychiatric disorders in a variety of ways. Since participants ranged in age from 23 months to 6 years of age, it is also possible that the relation between physical size and psychiatric symptoms depends on the age of the child.

Psychosocial dwarfism, which is a condition brought on by physical or emotional abuse or neglect is believed to be due, in part to, the effects of chronic activation of the HPA axis on growth processes. Although researchers have not investigated the HPA activity of children with psychosocial dwarfism, there is reason to believe that the condition is brought on by HPA hyper-activity due to the stressful environment (Charmandari et al., 2003). Children with psychosocial dwarfism show decreased GH levels when they are in the abusive or neglectful environments, but once they are placed into safe and nurturing environments, the GH levels return to normal (Albanese et al., 1994; Skuse et al., 1996). Evidence from exposure to HPA hormones in utero also substantiates the link between HPA activity and growth. Exposure to elevated levels of CRF and ACTH in utero has been linked to small-for-gestation-time infants (Charmandari et al., 2003).

The effect of caregiving on the growth system has also been demonstrated in non-human primates. Rhesus monkeys that were separated from their mothers at birth and nursery-reared for the first month of life showed lower basal GH levels than mother-reared subjects even though the

bottle-fed nursery-reared monkeys showed higher weights than mother-reared monkeys. This alteration was also linked to HPA activity, with nursery-reared infants demonstrating higher basal cortisol levels than controls (Champoux, Coe, Schanberg, Kuhn & Suomi, 1989). A study of caregiver-infant relationship disruption in another nonhuman primate, the common marmoset (*Callithrix jacchus jacchus*), revealed that differences in parental care during the first 3 weeks of the infant's life impacts growth and HPA activity during the juvenile period. The frequency that the infant was carried and groomed during the first 3 weeks of life was positively associated with body weight during the juvenile period prior to puberty. Infant abuse, which was described as biting of the infant's tail that resulted in serious wounds, was observed in a few family groups during the first 2-3 weeks of the infant's life. Abused infants had lower body weights as prepubescent juveniles and smaller knee-heel length and head-tail length around the time of puberty than non-abused infants.

Conclusions

From this review it is clear that in rats, and human and non-human primates, adequate caregiving is critical to the development of the neuroendocrine system. It is also clear that there is an association between the neuroendocrine system and behavior. However, the effects of caregiver-infant relationship disruption are complex. Age, psychiatric status in humans, type of maltreatment or relationship disruption, all need to be considered when attempting to understand the effects of this early trauma on neuroendocrine and behavioral measures.

APPENDIX D

LITERATURE REVIEW REFERENCES

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