THE EFFECTS OF MATERNAL MALTREATMENT ON THE BEHAVIORAL AND NEUROENDOCRINE DEVELOPMENT OF THE RHESUS MACAQUE (*MACACA MULATTA*) INFANT: THE FIRST SIX MONTHS

by

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(Under the Direction of Irwin Bernstein)

ABSTRACT

Across many species, early adverse experiences can alter the social and physiological development of individuals. The quality of the early caregiver-infant relationship can serve as a major source of vulnerability in later life. Evidence suggests that the hypothalamic-pituitary-adrenal (HPA) axis may be one system particularly vulnerable to the effects of early post-natal experience. Post-natal experiences can also impact early and later psychological development. This study examined the behavioral and neuroendocrine development of 10 maternally maltreated and 10 control rhesus macaques during the first six months of life. Mother-infant pairs were focally observed every week in captive groups at the Yerkes Field Station. In addition, reactivity ratings were also collected during times of stress. To obtain cortisol measures, basal blood samples were collected monthly, as well as blood samples following mother-infant separations during months 3 and 6. Compared to control infants, maltreated infants broke contact with their mothers less often, and they screamed more and exhibited more tantrums. Abusive mothers broke contact and rejected their infants more frequently than non-abusive mothers. Abused infants did not differ from control infants in their interactions with others. During the first month, when abuse rates were highest, abused infants had elevated levels of basal a.m. cortisol, in comparison to control infants. After the first month, however, they consistently exhibited lower basal and post-separation levels of cortisol compared to the control group. During stressful procedures, abused infants were less likely to be on their mothers, and they were less reactive to being handled. The data suggest that abused infants received lower quality care from their mothers, and that this impacted their cortisol levels. The lower levels of cortisol after month 1 are congruent with the reactivity data, suggesting that the behavioral and physiological systems of the abused infants may have become hypo-responsive in response to early adversity, thereby exhibiting blunted behavioral and cortisol responses. This suggests that rhesus monkeys exposed to early adversity may exhibit outcomes similar to those of maltreated humans, and that their neuroendocrine development may look similar to that of individuals with psychological disorders.

INDEX WORDS: Macaca mulatta, infant abuse, adverse experience, developmental outcomes, hypothalamic pituitary adrenal (HPA) axis, cortisol

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CHAPTER 1

INTRODUCTION

Over the past 20 years, compelling evidence has accumulated indicating that early adverse experiences, such as child maltreatment, result in an increased risk for the development of behavioral, mood, and anxiety disorders. The quality of the early caregiver-infant relationship can serve as a major source of vulnerability in later life. Evidence from animal studies suggests that the hypothalamic-pituitary-adrenal (HPA) axis may be one system particularly vulnerable to the effects of early post-natal experience. Post-natal experiences can also impact early and later psychological development. Only recently have we begun to understand the mechanisms behind how early experience impacts these two systems.

The activation of the HPA axis, as well as the sympathetic-adrenomedullary system, has traditionally been regarded as the primary endocrine response of mammalian organisms to stress (Selye, 1975). As the individual perceives a stressor, information is transmitted and integrated in the hypothalamus (specifically the paraventricular nucleus), which secretes the neuropeptide corticotropin-releasing factor (CRF) into the hypophyseal portal vascular system. The release of CRF stimulates the production of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH binds to receptors on the adrenal cortex, which stimulates the secretion of glucocorticoids or CORT (corticosterone in the rat, cortisol in the primate) from the adrenal cortex into the system. CORT operates to increase energy available for action by inhibiting glucose uptake into storage sites and liberating energy from fat and protein sources (Gunnar & Davis, 2003). It also stimulates cardiovascular and pulmonary function to support the increased motor activity needed in times of stress. The system is able to shut down, via a negative feedback loop. The increased levels of circulating CORT bind to glucocorticoid receptors on the pituitary, hypothalamus, and in the limbic system (Ladd et al., 2000). This inhibits the further production of CRF, ACTH, and CORT. The HPA axis exhibits a circadian cycle, with a peak of CORT around the time of waking and a decline in levels throughout the rest of the day.

The HPA axis matures and becomes organized over the course of pre- and post-natal development (Walker, Anand, & Plotsky, 2001). It is likely that pre- and postnatal experiences play a critical role in the development of stress reactivity and regulation (Heim, Owens, Plotsky, & Nemeroff, 1997). Early rearing seems to be critical to the normal development of the HPA axis. Postnatal disruption of normal maternal-infant interactions results in the dysregulation of the developing HPA axis, and ultimately maladaptive changes in the developing neuroendocrine stress response in rodents and primates (Sanchez, Ladd, & Plotsky, 2001).

Many studies (see Appendix A) have reported that children with a history of abuse were more anti-social, disruptive, and aggressive compared to non-abused children (Alessandri, 1991; Dodge, Bates, & Pettit, 1990; Fagot, Hagan, Youngblade, & Potter, 1989; George & Main, 1979; Kaufman & Cicchetti, 1989). Other have also reported that young physically abused children initiated fewer positive interactions with others, displayed avoidance behavior, and had a higher number of negative interactions compared to non-abused children (Haskett & Kistner, 1991; Jacobson & Straker, 1982). Resting levels of cortisol and ACTH have been reported to be lower in children and adults that had experienced abuse and/or neglect, without a secondary diagnosis of post-traumatic stress disorder (PTSD) (Bruce, Kroupina, Parker, & Gunnar, 2000; Cicchetti & Rogosch, 2001; DeBellis et al., 1994; Gunnar & Vazquez, 2001; Heim, Newport, Bonsall, Miller, & Nemeroff, 2001). Maltreated children also demonstrate elevated levels of cortisol in response to stressors many years later (Gunnar, Morison, Chisolm, & Schuder, 2000). Heim et al. (2000) found that women who had been abused as children had higher levels of ACTH and cortisol in response to a stress test compared to control women, and that this effect was magnified when the women had a diagnosis of depression. The HPA circadian rhythm of maltreated children also deviates from that of non-abused children (Cicchetti & Rogosch, 2001; Hart, Gunnar, & Cicchetti, 1995) (See Appendix A for discussion).

Further information regarding the development of the HPA axis under normal and adverse rearing conditions can be found in the animal literature (see Appendix B and C). Peer-reared rhesus macaque infants were more hostile and aggressive and showed less sexual behavior and play with peers compared to mother-reared infants (Ruppenthal, Arling, Harlow, Sackett, & Suomi, 1976). Clarke (1993) found that peer-reared infants had lower plasma cortisol and ACTH levels later in life compared to mother-reared infants. When exposed to repeated separations from their attachment figure, peer-reared animals had higher basal and post-stressor plasma cortisol across the first two years compared to mother-reared infants (Higley, Suomi, & Linnoila, 1991). However, other researchers found larger cortisol responses in response to separation in maternally reared animals (Clarke, Kraemer, and Kupfer, 1998; Shannon, Champoux, and Suomi, 1998). Nursery reared infants have also been found to exhibit a shifted peak in their resting morning cortisol levels (Boyce, Champoux, Suomi, & Gunnar, 1995).

Only a few studies have examined the behavioral outcomes of maternal maltreatment in non-human primates. Maestripieri and Carroll (1998) examined the effects of maternal maltreatment in pigtail macaques (*Macaca nemestrina*). Abusive pigtail mothers had controlling parenting styles, meaning that they rejected their infants and protected them more than non-abusive pigtail mothers (Fairbanks, 1996). Compared to control infants, the abused pigtail infants demonstrated less play at a later age, and initiated play bouts less often. Maestripieri, Jovanovic, and Gouzoules (2000) also found that abused rhesus infants were slower at gaining independence from their mothers, and spent more time on their mother during the first two months of life.

These outcomes suggest that early stressors imposed on the developing primate can have profound effects on behavior and the neuroendocrine system. The lack of maternal contact and/or a normal mother-infant relationship may disrupt the development of the HPA axis, as well as prevent the acquisition of appropriate social skills (Sanchez et al., 2001). Likewise, many of the disruptions in the HPA axis just described are identical to those found in patients with PTSD. PTSD patients demonstrate higher than normal levels of basal CRF in their cerebrospinal fluid (Yehuda, 1998). However, many, but not all, PTSD patients have lower than normal basal levels of cortisol (see Liberzon, Abelson, Flagel, Raz, & Young, 1999 for conflicting findings), and they have a blunted cortisol response during times of stress (Yehuda, 1998). After exposure to a a non-specific stressor, PTSD patients return to resting levels of cortisol sooner than non-PTSD individuals (Yehuda, Giller, Levengood, Southwick, & Siever, 1995). These patients demonstrate a flattened circadian rhythm (Gunnar & Davis, 2003; Yehuda, 1998). Thus, many of the physiological responses to early stressors observed in humans and non-humans from adverse backgrounds may be the precursors for future anxiety disorders.

John Bowlby's theory of attachment and Gary Kraemer's psychobiological attachment theory (PAT) also help explain the alterations observed in maltreated individuals (see Appendix D for a review of the two theories). Attachment theory holds that infants become securely attached to caretakers who consistently and appropriately respond to their needs (Bowlby, 1958, 1969, 1973, 1980). Secure infants are confident that their caretakers will meet their needs, and they feel safe to examine their environment. Insecure children do not feel protected by their caretakers, and are less likely to leave their caretakers and explore. When securely attached infants are stressed, they quickly seek their attachment figure (Ainsworth, Blehar, Waters, & Wall, 1978). These infants are easily calmed and reassured by their caretaker, and are ready to explore their environment again. Insecurely attached infants may seek the mother when stressed, or they may behave ambivalently towards her. They often fail to be comforted by the mother, and are more reluctant to explore their environment after periods of stress.

Psychobiological attachment theory posits that the regulation of the infant's physiology is not only a result of genetic endowment, but also due to maternal regulation (Kraemer, 1997). According to PAT, the mother provides an external regulatory mechanism for the infant's systems, because the infant cannot regulate all systems on its own right after birth. The neurobiological systems of the infant develop in sequence and in relation to the caregiver, and the infant cannot respond competently until these systems are in place (Kraemer, 1992). The regulatory capabilities of the infant can be compromised if the caregiver fails to provide an adequate physiological model for the infant (Kraemer, 1992).

The above evidence suggests that early adverse experiences can have deleterious effects in humans and non-humans. Early stressors imposed on the developing human and non-human primate can also have profound effects on behavior and the neuroendocrine system. Infants exposed to stressors may have difficulty interacting with others later in life, and may have problems regulating their emotions. They may also exhibit irregularities in their resting levels of cortisol, in their response to stressors, and in their HPA circadian rhythm. Early adversity also appears to be a primer for future psychopathology. Unfortunately, the data in this field are by no means consistent. However the research does suggest that there are strikingly similar behavioral and neuroendocrine outcomes in human and non-human primates from adverse backgrounds. This demonstrates that animals can be used to provide further insight into the effects of early adversity on the developing individual. The research also indicates that, across several species, abnormalities in the mother-infant relationship usually result in individuals with some degree of psychological and/or neuroendocrine deficits.

The purpose of the present research project was to evaluate whether maternally abused rhesus macaque infants exhibit similar behavioral and neuroendocrine outcomes as do human children, and to determine developmentally when the effects of abuse would be evident. It was predicted that the relationships between abused infants (those that were dragged, crushed, thrown, stepped/sat on, rough groomed, or awkwardly carried by their mothers) and their mothers would be quantitatively different than the relationships between non-abused infants and their mothers. Based on attachment theory, I predicted that the altered relationship between abused infants and their mothers would cause disruptions in the infants' relations with others. Specifically, it was predicted that, compared to non-abused infants, abused infants would spend more time on their mothers, and less time with other animals. Abused infants were predicted to engage in less contact with other animals, less social and solitary play, and to receive and direct more agonistic actions towards others (attack, avoid, chase, grimace, harass, threat) compared to non-abused infants. Again, this was based on attachment theory's premise that early alterations in the mother-infant relationship would cause alterations in the infant's later relationships. Previous research (Maestripieri, 1998) has noted that abusive mothers also differ in their care-giving styles compared to non-abusive mothers. I also predicted that abusive mothers would demonstrate differences in levels of restraint, rejection, grooming, and ventral contact with their infants, compared to non-abusive mothers. I also expected that abused infants would have higher rates of nervous behavior (scratching, yawning, body-shaking, self-grooming) compared to control infants because of the strained relationship with their mothers. Compared to control infants, abused infants were predicted to have higher rates of screaming, cooing, and tantrums. This was based on the assumption that the abusive mothers would not be as responsive to their infants' needs as control mothers, and that the infants would react to their needs not being met with increased vocalizations.

Predictions were also made regarding how the infant and its mother would respond behaviorally during stressful procedures. The attachment literature suggests that during stressful times secure and some insecure infants seek proximity and contact with their mothers (Ainsworth et al., 1978). However, there is also evidence that during stressful times, insecure infants may seek proximity to their mothers, but not direct contact (insecure-avoidant) (Ainsworth et al., 1978). Thus, it was predicted that if any animals were to be off of their mothers during these procedures, it would be the abused infants. If it were the case that the abused infants' HPA axis' were regulating differently than the control infants, I predicted that there would also be differences in how they reacted to being handled. Levine, Franklin, and Gonzalez (1984) found that behavioral and physiological reactivity do not always correspond with one another. Thus, if the HPA axis were hyporesponsive, the infant could be highly reactive or not reactive at all. Therefore I predicted non-directional differences between the abused and non-abused infants in how they reacted to being handled.

Another aim of this project was to assess attachment security through several testing paradigms. In their natural environment, insecurity would be demonstrated in infants who, remained in contact with their mothers for extended periods of time, continually made contact but seldom broke contact with their mothers, and engaged in less social play with others. This would be expected of insecure infants after three months of age, when most infants spend a large percentage of their time away from their mothers, exploring their environment (Hinde & Spencer-Booth, 1967). During stressful conditions insecurity was classified as infants who did not remain on their mothers (although, this does not imply that all infants who remain on their mothers are securely attached). It was also expected that during conditions in which secure infants were calm, they would explore their surroundings, while insecure infants would be expected to remain on their mothers and not explore their surroundings. By examining the infants across these different conditions, I expected to observe insecure behavioral patterns among the abused infants and secure behavioral patterns among the non-abused infants.

PAT holds that alterations in the mother-infant relationship can affect the physiological development of the infant. I therefore predicted that abused infants would demonstrate alterations in their HPA axis (as measured by cortisol levels). Compared to control animals, I expected abused infants to have higher basal levels of cortisol at month 1 as a function of the abnormal mother-infant interactions during the first month. It was expected that the HPA axis of the abused infants would respond to the early abuse, and that the system would demonstrate different levels of cortisol at months 2, 3 and 4 compared to animals that had not been exposed to abnormal mother-infant interactions. If the HPA axes of the abused infants were still responding to the abnormal mother-infant relationship, then they would have higher resting and post-stressor levels of cortisol compared to control infants. However, if the HPA axis of the abused infants was beginning to down-regulate to accommodate for the increased levels of cortisol due to the altered mother-infant relationship at month 1, then the abused infants would have lower resting and stress-response levels of cortisol compared to the non-abused infants. It was also predicted that abusive mothers would not be able to buffer the stress response of their infants as well as control mothers at month 2. In a testing paradigm termed “maternal buffering” Levine, Johnson and Gonzalez (1985) reported that when a monkey mother and her infant are placed in a novel environment together, the infant rarely demonstrated elevations in cortisol. The mother provided a buffer to the stressful experience. Thus it was predicted that control infants would not demonstrate as elevated a cortisol response as abused infants when placed in a novel environment with their mothers. This was based on PAT, which would predict that the altered mother-infant relationship in the abusive mother-infant pairs would impact the development and responsiveness of the infants’ physiological systems. Securely attached infants would have mothers that buffered the infants’ stress response, while insecurely attached infants would not.

By six months of age, infants would not be as dependent on their mother as they were from months 1-3. Because six-month old infants spend a lot of time off of their mothers, it was expected that their HPA axis would be regulating on its own (i.e. it will not be in a state of transition and development as it was earlier). I predicted that basal and post-stressor levels of cortisol would be lower in the abused infants compared to the control infants. This prediction was based on the data in humans and primates which suggests a hypo-functional system after exposure to early stressors (Bruce et al., 2000; Clarke et al., 1998; Coplan et al., 1996; DeBellis et al., 1994; Hart et al., 1995; Heim et al., 2000; Shannon et al., 1998), as well as PAT which would predict irregularities in the biological systems of animals exposed to early post-natal stress.

It was expected that the irregular relationship between abused infants and their mothers would lead to irregularities in the developing biological systems. Specifically, if the relationship was not typical, the mother would not be able to entrain her infant to her internal circadian rhythm as suggested by Suomi (1999). Several studies have demonstrated that individuals exposed to early stressors have a flattened circadian rhythm (Hill, McCormack, & Mason, 1973; Bruce et al., 2000; Gilles, Berntson, Zipf, & Gunnar, 2000). It was therefore predicted that abused infants would have a flattened circadian rhythm across the day, with a smaller morning peak, and less change in cortisol across the day compared to non-abused infants.

CHAPTER 2

METHODS

Subjects

Subjects were 20 rhesus macaque infants living with their mothers in four large social groups at the Field Station of the Yerkes National Primate Research Center in Lawrenceville, GA. Each group consisted of two or three adult males and 18-49 adult females with their sub-adult and juvenile offspring (see Table 1 for group compositions). Food and water were freely available to all animals.

Prior to the 2002 birth season, fifteen abusive mothers were identified from their history reports that indicated that they physically abused most, if not all of their prior offspring. Maternal abuse was defined as the occurrence of at least three instances of the following actions directed towards the infant (see Table 2 for full definitions): 1) dragging, 2) crushing, 3) throwing, 4) stepping or sitting on, 5) rough grooming, and 6) abusive carrying. Biting and hitting were not classified at abusive behaviors because they often occur in the context of punishment and weaning.

Approximately twenty non-abusive mothers were identified as possible controls. All animals were given ultrasonic examinations to determine pregnancy and expected delivery dates. Around each animal's expected delivery date, she was observed daily for the birth. The sex of the infant was identified on the second day of life for each infant.

One of the fifteen abusive mothers maltreated her infant so severely that it had to be removed from her. Two of the abusive mothers killed their infants before they could be

Table 1: 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

Table 2: Ethogram of rhesus macaque behavior (adapted from Maestripieri, 1998)

Name	Definition
attack	physical assault by an animal
avoidance	turning or moving away when an animal approaches within 1 meter
bite/hit	common definition
body shake	shaking fur like a wet dog
chase	pursuit in agonistic context, not in play
contact	body contact initiated
end contact	body contact terminated
on ventrum	infant is in ventral contact with mother
off ventrum	ventral contact ends
on dorsal	infant is on dorsum of mother
off dorsal	dorsal contact ends
other contact	infant is in any contact with mother, other than ventral or dorsal
off other	other contact ends
cradle	ventro-ventral contact with the other animals arms wrapped around infant
end cradle	cradle terminated
grimace	corners of the mouth are pulled back, lips are parted, and teeth are closed
groom	picking and spreading fur of another animal
end groom	grooming terminated
harass	pulls, drags, or hits another
reject	prevent contact or infant access to nipple by holding the infant at a distance with an arm, passively blocking the chest with an arm, or twisting torso away
restrain	actively prevent infant from breaking contact by pulling its leg or tail
scratch	common definition
social play	rough and tumble, chase play with another animal
end social play	social play terminated
solitary play	play with objects, or running, spinning, and gamboling
end sol play	solitary play terminated
tantrum	infant's body shakes, while infant screams
threat	open mouth, stare with/without vocalization
vocalize	screams or coos (no body movement)
yawn	common definition
Abusive Behavior	
drag	drags infant while walking or running
crush	pushes infant against the ground with hands
throw	throws infant ahead while walking or sitting
step on/sit on	steps or sits on infant
rough groom	forces infant onto the ground, and pulls out infant's hair with force
abusive carry	carries infant with one arm away from her body, infant unable to cling

removed. Three previously abusive mothers did not abuse their infants during the 2002 year. This left nine abusive mother-infant pairs. A tenth previously abusive mother was added to the study after she was observed to abuse her 2002 infant during the first week of life. Each abused infant was matched with a non- abused infant on the following criteria: age (within one month), sex, and whenever possible, social group and the mother's dominance rank (see Table 3). As expected, none of the non-abusive mothers were observed to abuse their infants. There were a total of six abused female infants, and four abused male infants. Infants were born between March 19, 2002 and July 15, 2002.

Apparatus

Animals were housed in 38 x 38 meter outdoor compounds with an attached 3.2 x 11.2 meter indoor area. Animals had access to the indoor area via two doors. The animal's indoor unit had a guillotine door used to run the animals into transport boxes. Inside the building were .9 x .6 x .6 meter squeeze cages in which the animals were placed for handling.

A 5-meter tall observation tower was situated at the corner of each compound, and was equipped with electricity, tables, and chairs for data collection. This location provided an unobstructed view of the compound.

Behavioral data was collected using binoculars and a handheld computer (Palm Pilot IIIXE) programmed to allow the recording of the frequency, duration and sequence of behavior. The definitions of behavioral categories that were collected are shown in Table 2.

Blood samples were collected using 1 ml syringes and 23-gauge needles. They were collected in pre-chilled EDTA plastic tubes, centrifuged at 3000 rpm for 12 minutes at 4ºC, and plasma samples were aliquoted and stored at –80ºC until assay. All plasma samples were

Table 3: 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

assayed for cortisol using commercially available radioimmunoassay (RIA) kits (ICN Pharmaceuticals, Costa Mesa, CA).

Procedure

Three experienced observers collected the data. For reliability purposes, prior to the beginning of data collection, observers watched and recorded behavior until percent agreement exceeded 90% and Cohen's Kappa exceeded 0.8.

Focal observations began on the second day of life for each infant. Each mother-infant pair was observed for a 30-minute period, five times per week, during the first month of life, two times per week during the second month of life, and one time per week from the third month through the sixth month of life. Observations were done between 7 a.m. and 11:00 a.m., when the animals were most active. All animals were locked outdoors during observation sessions.

The following measures were calculated from the data to quantify the mother-infant relationship: 1) proportion of time spent in contact, 2) proportion of time the mother spent cradling the infant, 3) proportion of time the mother spent grooming the infant, 4) proportion of time spent that the mother and infant were in ventral-ventral contact, ventral-dorsal contact, and any other contact, 5) frequency of contacts made and broken by the mother and the infant, 6) frequency of maternal rejections, 7) frequency of maternal restraints, and 8) frequency of abusive episodes. The following measures were taken to quantify the interactions between the infant and others: 1) frequency and duration of contact initiated and received by the infant, 2) frequency of agonistic interactions with others (attack, avoid, bite, chase, grimace, harass, hit, threat), and 3) frequency and duration of social play. The following measures were taken to quantify self-directed infant activity: 1) frequency of nervous activity (yawning, scratching, self-grooming), 2) frequency of vocalizations (screams, coos) and tantrums, 3) frequency and duration of solitary play.

Subjects were captured every month during the first six months of life to obtain blood samples and to undergo physiological testing. All mothers had been previously trained to run into the capture area (indoor area) with their infants, where they could be transferred into a squeeze cage via a transfer box. Once in the squeeze cage, the infant was removed from the mother and a blood sample was taken from the femoral vein without anesthesia within 5-10 minutes of researchers entering the building. All morning samples were collected at sunrise, and the evening sample was collected at sunset.

The following time line was used for the administration of the physiological tests and collection of blood samples from the infants:

Month 1: Basal blood sample: During the fourth week of life, each infant and mother were captured and one 0.5 ml sample of blood was obtained from the infant in the morning. The infant was immediately returned to its mother and released to the group.

Month 2: Basal blood sample, maternal buffering test, post maternal buffering blood sample: During the eighth week of life, each mother-infant pair was captured and one 0.5 ml sample of blood was obtained from the infant in the morning. The infant was immediately returned to its mother and released to the group. During the ninth week of life, each mother-infant pair was captured and placed in a squeeze cage in a novel room for 30 minutes. At the end of this 30-minute separation period, the infant was removed from the cage, and another 0.5 ml sample of blood was taken. The infant was immediately returned to its mother and released to the group.

Month 3: Basal blood sample, separation test, post-separation blood sample, 2-hour reunion, post-reunion blood sample: During month 3, the infants and their mothers were captured and one

0.5 ml basal sample of blood was taken from the infant in the morning. Basal blood samples were obtained from the infant two times during month 3 (one time for this separation test, and another for a different test not discussed here). The infant and mother were then transported to two separate rooms, and each was placed alone in a squeeze cage for 30 minutes. At the end of this 30-minute separation period, the infant was removed from the cage, and another 0.5 ml sample of blood was taken. The infant was then returned to the mother in a squeeze cage for a two-hour reunion period. Each infant was tape-recorded during the separation and the reunion. The last 30-minute period of each tape was coded for mother-infant behavior (the definitions of behavioral categories that were coded are shown in Table 4). At the end of this two-hour reunion, the infant was again captured, and a 0.5 ml sample of blood was taken. The infant was returned to its mother, and the animals were returned to their social group.

Month 4: Basal blood samples (circadian rhythm): During weeks 16 and 17, each mother-infant pair was captured and one 0.5 ml basal sample of blood was taken from the infant, once in the morning, and once in the evening (the order of these captures was counter-balanced). The infant was immediately returned to its mother and both were released into the group.

Month 6: Basal blood sample, separation test, and post-separation blood sample: During the sixth month of each infants life, each infant and its mother were captured and one 0.5 ml basal sample of blood was taken from the infant in the morning. The infant and mother were then transported to two separate rooms, and each was placed alone in a squeeze cage for 30 minutes. At the end of this 30-minute separation period, the infant was removed from the cage, and another 0.5 ml sample of blood was taken. The infant was then returned to the mother, and the animals were returned to their social group.

Table 4: Ethogram of mother and infant behavior during two-hour reunion

Name	**Definition**
contact	body contact initiated between mother and infant
end contact	body contact terminated
explore	oral or tactile investigation of surroundings; calm examination of environment; not frantic or aggressive manipulation
end explore	exploration ends
agitation	rapid, jerky movements; animal is shaking cage or bar violently, biting cage or bar, frantically throwing arm out
end agitation	agitated behavior ends
freeze	animal remains tensely still, body is stiff, eyes are focused
end freeze	freezing ends
locomotion	animal moves from one quadrant to another
nervous behavior	yawn, scratch, bodyshake
reject	mother prevents infant contact or access to nipple by holding the infant at a distance with an arm, passively blocking the chest with an arm, or twisting torso away
restrain	mother actively prevents infant from breaking contact by pulling its leg or tail
resting/passive	animal remains still, but calm, and not scared; can be sitting or hanging calmly (infant must be off of mother)

During each of the animal captures, throughout the first four months of life, each mother-infant pair was rated by one of the researchers. Mothers and infants were rated on two dimensions:1) reactivity to the stressful situation of being captured and handled, and 2) how close the mother and infant were during the process. The scales used for the mothers and infants can be found in Tables 5 and 6, and were adapted from a temperament scale created by Schneider and Suomi (1992).

Attachment quality of the abused and non-abused infants was assessed by collectively examining the infant behavioral data collected while the infant was in the home environment, the mother-infant data scored during capture, and the infant behavior scored during the last 30-minutes of the reunion following separation at month 3. Abused and non-abused infants were compared on the following: 1) proportion of time spent on the mother when in the home environment, under non-stressful conditions, 2) frequency of infant contact makes and breaks towards the mother in the home environment, 3) proportion of time infant spent engaged in social play, 4) proportion of time spent on the mother during the last 30 minutes of the post-separation reunion, 5) proportion of time spent exploring during the last 30 minutes of the post-separation reunion, and 6) how close the mother and infant were across the first four months when they were being captured for blood draws (as measured by the assessment scales). The abused and non-abuse animals had to differ from one another on all of these measures for conclusions about attachment security to be made.

Data Analysis

Behavioral data was analyzed in terms of the frequency or duration of behavior per 30 minutes, and averages were calculated for each postnatal month. Maternal-infant interactions, infant-other interactions, and infant self-directed behavior were analyzed with 2 x 6 repeated

Table 5: Infant assessment scale

1. Predominant state in capture unit:
    - 0=alert, awake, and aware
    - 1=alert, but somewhat agitated
    - 2=extremely agitated (body jerks and screams)
    - 3= freezing behavior, hanging from mesh
2. Infant “attachment” level to mom in capture unit
    - 0=on mom
    - 1=50% of time on mom
    - 2=off mom
3. Resistance to tester’s hold (before and after blood draw):
    - 0=no resistance (passive or compliant)
    - 1=moderate resistance
    - 2=constantly resists tester’s hold
4. Degree to which infant clings to tester before and after blood draw:
    - 0=passive/no cling
    - 1=moderate cling
    - 2=moderate cling with slight grasps and releases
    - 3= tight cling or frantic intermittent grasps and releases
5. Infant’s response during blood draw:
    - 0=no resistance (passive or compliant)
    - 1=moderate resistance
    - 2=constantly resists tester’s hold
6. Ease with which infant was consoled or calmed:
    - 0=not necessary to console
    - 1=easy to console
    - 2=consoles with difficulty
    - 3=cannot be consoled
7. Occurrence of distress coos or screams
    - 0=no obvious distress vocalization
    - 1=one coo or one scream
    - 2=2 coos, 3 or fewer screams
    - 3=many coos and/or screams
8. Occurrence of body jerks or tantrums
    - 0=no obvious jerks or tantrums
    - 1=one body jerk or tantrum
    - 2=two to four body jerks or tantrums
    - 3=many body jerks or tantrums
9. Predominant state after assessment, while holding
    - 0=alert, awake, and aware
    - 1=alert, but somewhat agitated
    - 2=extremely agitated (body jerks and screams)

Table 6: Mother assessment scale

1. Predominant state in capture unit:
   - 0=relaxed
   - 1= somewhat agitated
   - 2=extremely agitated (body jerks and screams)
   - 3= frozen, hanging from mesh; difficult to transfer to box
2. Predominant state in squeeze cage;
   - 0=holding infant tightly
   - 1=infant on and off of mother
   - 2=does not hold infant
3. Initial reaction of mother to testers attempt to get infant (in cage):
   - 0=not distressed at infant removal
   - 1=moderately distressed, mild attempts to prevent removal
   - 2=very distressed, bites/grabs at tester, holds infant tightly
4. Mother's response during blood draw:
   - 0=no resistance (passive or compliant)
   - 1=moderate resistance
   - 2=constantly resists tester's hold
5. Occurrence of distress calls to infant
   - 0=no obvious distress vocalizations
   - 1= one vocalization (screams, grunts)
   - 2=two to four vocalizations
   - 3=many vocalizations
6. Response of mother to return of infant;
   - 0=takes infant immediately, holds it tightly
   - 1=takes infant immediately, but does not hold it tightly
   - 2=does not take infant immediately
   - 3= rejects, hits infant

measures ANOVAs. The factors were maternal style (abusive and non-abusive) and time (6 months). Each ANOVA examined the main effects for time and abuse, as well as the interaction of time and abuse. When the assumption of sphericity was violated, the corrected Huhn-Feldt statistic was used. Significant findings for group by time interactions were followed by Bonferroni corrected dependent and independent t-tests (time main effects were not a focus of these analyses). A total of 11 ANOVAs were calculated (see Table 7 for a listing of all comparisons), thereby inflating the family-wise error rate. According to the binomial distribution, the probability of obtaining one or two significant outcomes by chance is greater than .05, whereas the probability of obtaining three significant outcomes by chance is .014. Therefore, a minimum of three tests needed to be significant to conclude that there were behavioral differences between the abused and non-abused animals.

Levels of basal cortisol (months 1, 2, 3, 4, 6) were compared between the two groups across the four samples using a repeated measures 2 x 5 ANOVA. Because two basal samples were collected at month 3, the average of the two samples was used for this analysis. When the assumption of sphericity was violated, the corrected Huhn-Feldt statistic was used. A priori planned comparisons were made using uncorrected t-tests. Post-hoc comparisons were made using Bonferroni corrected dependent and independent t-tests.

At month 2, differences scores were calculated between the basal (T1) and the post-maternal buffering (T2) samples of cortisol. An independent t-test was run on the difference scores to determine if the increase in cortisol from T1 to T2 was different for the abused and non-abused infants.

At month 3, the two groups of animals were compared on their changes in cortisol levels from resting (T1) to post-separation levels (T2) to post-reunion levels (T3) with a 2 x 3 repeated

Table 7: Behavioral analyses

Behavioral Comparisons—2 (abuse vs. non-abuse) x 6 (6 months) ANOVAs, on each of the following variables:

1. Duration of total contact between mothers and infants
    - if there were significant differences between abused and non-abused infants, the differences in quality of contact (ventral, dorsal, and other), and initiator of contact were to be analyzed
2. Duration of grooming bouts between mothers and infants
3. Frequency of rejection from mother
4. Frequency of restraints from mother
5. Duration of contact between others and infants
6. Frequency of agonistic interactions (attack, avoid, chase, grimace, harass, threat) between infant and others
7. Duration of social play interactions with others
8. Frequency of nervous behavior (yawns, scratches, bodyshakes)
9. Duration of solitary play bouts
10. Frequency of vocalizations
11. Frequency of tantrums

measures ANOVAs. Again, the average of the two month 3 basal cortisol samples were used in these analyses. To determine if changes in cortisol from T1 to T2, and from T2 to T3 were significantly different for the two groups, difference scores were calculated and compared using Bonferroni corrected independent t-tests. At month 6, the resting and post-separation cortisol levels were compared using a 2 x 2 repeated measures ANOVA. Difference scores were again calculated and an independent t-test was used to determine if the change in cortisol differed between the abused and non-abused groups. Finally to determine if the stress response from resting levels (T1) to post-separation (T2) changed from month 3 to month 6, the difference scores at month 3 were compared to the difference scores at month 6, using a 2 x 2 repeated measures ANOVA.

The morning and evening cortisol samples that were taken during month 4 were used to calculate the two groups' circadian rhythm. A repeated measures 2 x 2 ANOVA was used to compare the two groups at each time point. To determine if change in cortisol across the day was different for the two groups, difference scores between morning and evening samples were calculated, and compared with an independent t-test.

The behavioral data that was collected from the reunion-tapes were analyzed with independent t-tests. The infant/mother assessment data that were collected during each capture was analyzed at each time period (month 1, 2, 3 and 4) using a non-parametric statistic for ordinal data, Gamma (γ). In order to assess security of attachment, behavioral data from the compound, the behavioral data during the month 3 reunion taping, and the mother-infant assessment data were qualitatively considered together in order to determine if abused infants would be more likely to exhibit insecure attachment behavior, and whether non-abused would be more likely to display secure attachment behavior.

CHAPTER 3

RESULTS

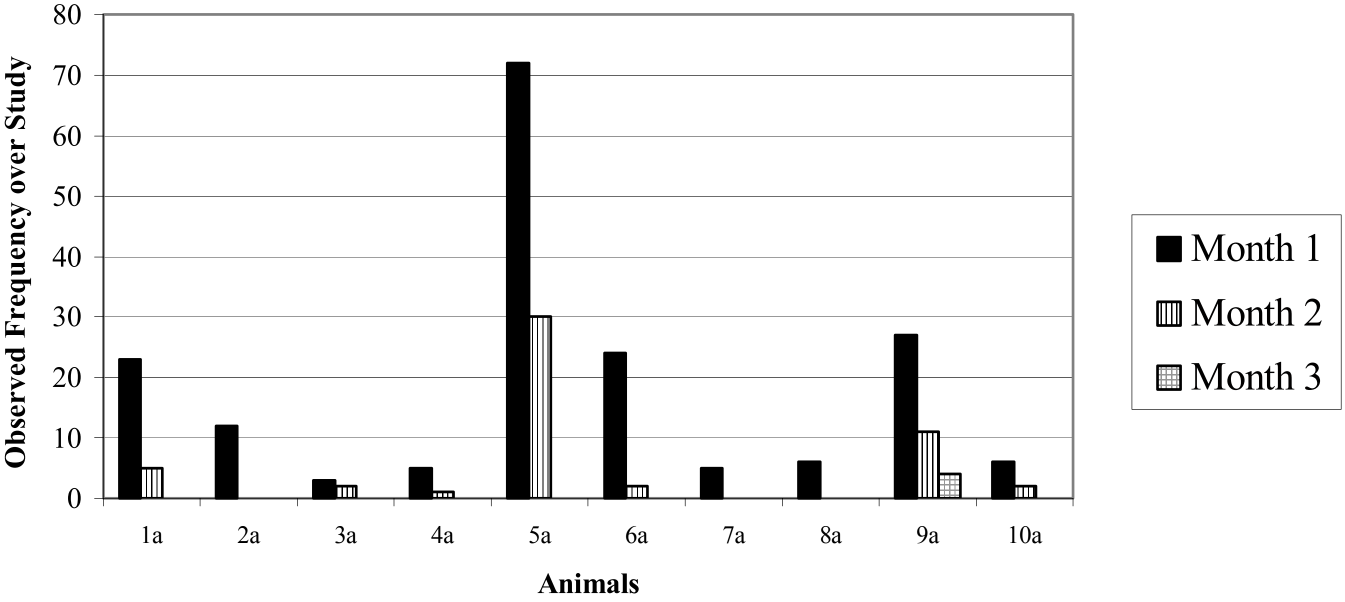
Behavioral Observations

Each mother in the abusive group mistreated her infant at least five times during the observational periods (see Figure 1). There was considerable variation in abuse counts over subjects (*M*=24.00, *SD*=30.19). Hourly rates of abuse over the three months ranged from .30 to 6.4. Figure 1 demonstrates that abuse was most frequent in month 1, and was infrequent by month 3. As expected, abuse was never observed in the control animals.

There was not a significant group effect for the proportion of time spent in contact between infants and their mothers, however there was a significant group by time interaction, $F(4.33, 77.91)=2.46$, $p<.05$ (see Figure 2). Six Bonferroni corrected t-tests were run to compare each group at each month. These tests failed to determine where the group by time interaction was occurring.

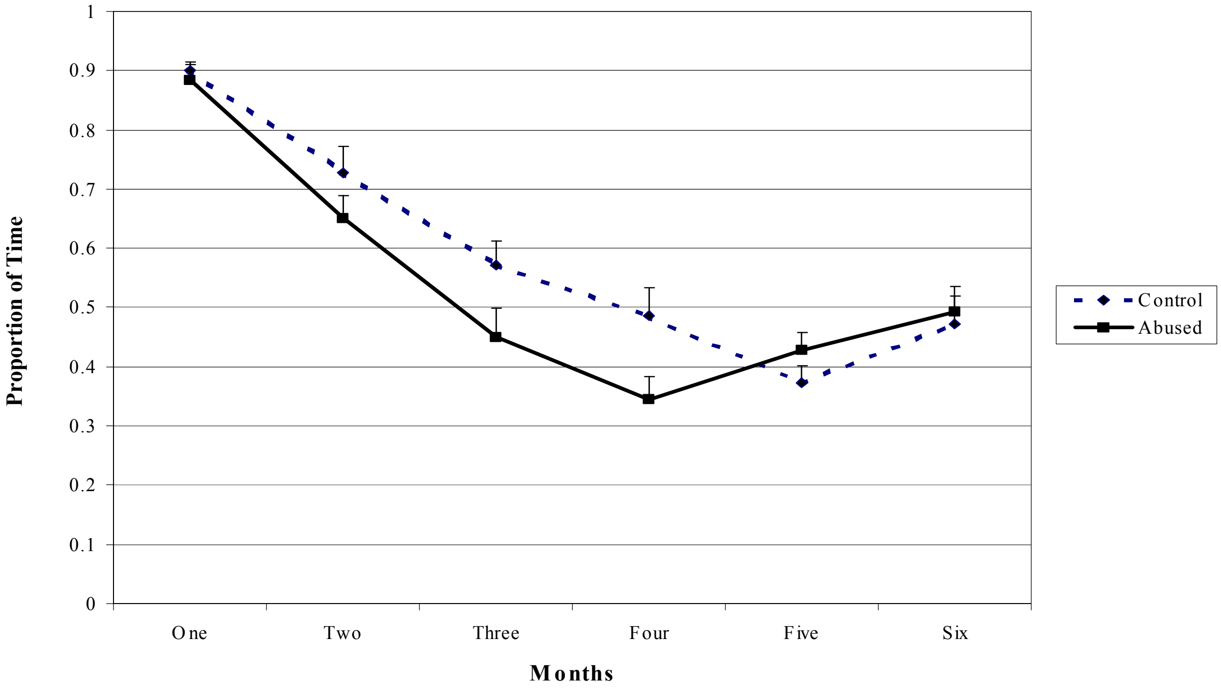
Because there was a significant group by time interaction for proportion of time spent in contact between mother-infant pairs, the quality of mother-infant contact was examined. There were no group main effects for proportion of time spent in either ventral or other contact. There was a significant group by time interaction for the proportion of time infants spent in ventral contact with their mothers $F(3.90, 69.36)= 2.77$, $p<.05$. This interaction looked identical to the one observed in Figure 2. Six Bonferroni corrected t-tests failed to determine where the group by time interaction was occurring. There was also a significant group by time effect for proportion of time spent in other contact between mother-infant pairs, $F(3.56, 64.15)=2.96$,

Figure 1: Frequency of abuse in the 10 abusive mothers



Note: Range of observed abuse: 5-102

Figure 2: Proportion of time spent in contact between infants and mothers



Note: Group x Time effect. Post-hoc tests not significant.

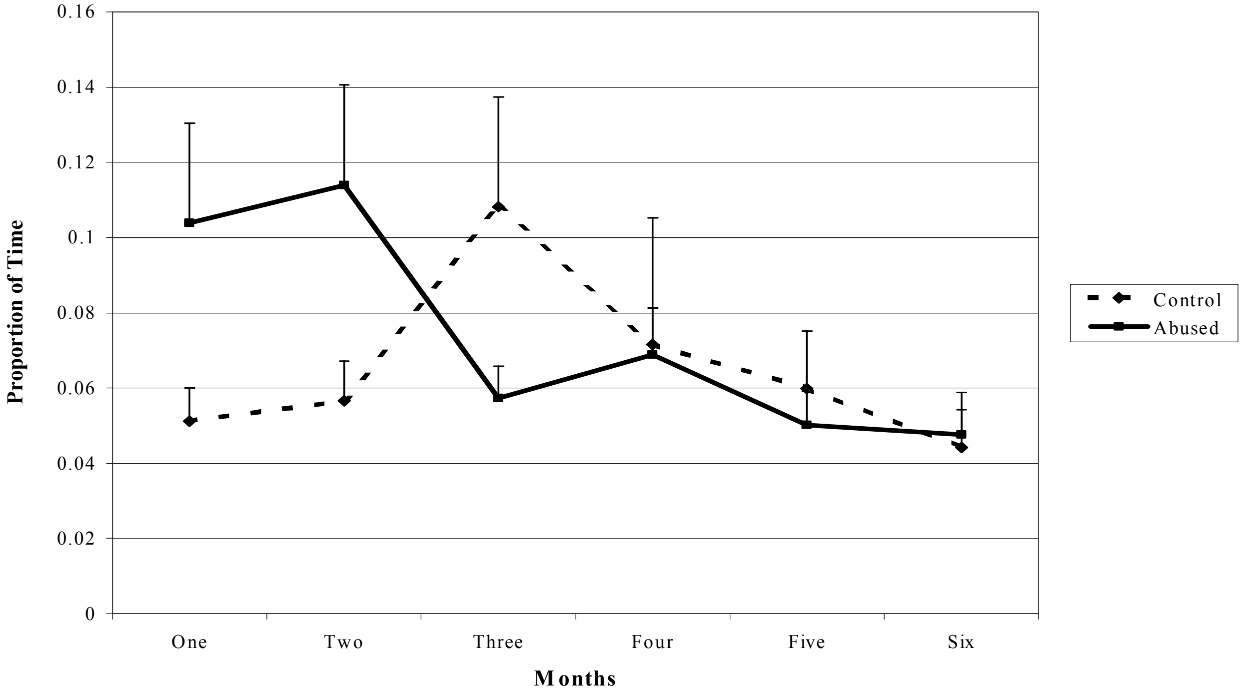
p<.05 (see Figure 3). Six Bonferroni corrected t-tests compared the two groups at each month. These tests failed to determine where the group by time interaction was occurring. There were no group or group by time effects for dorsal contact.

Abused infants (*M*=3.42, *SE*=.24) broke contact with their mothers less often than non-abused infants (*M*=4.46, *SE*=.13), *F*(1, 18)=14.72, *p*<.01 (see Figure 4). Across the six months, the average rate of break contacts appeared higher in abusive mothers (*M*=1.70, *SE*=.19) compared to control mothers (*M*=1.21, *SE*=.17), however the difference was not significant, *F*(1, 18) =3.41, *p*=.07 (see Figure 5). A sign test, however, indicated that the abusive mothers did break contact more often, *p*<.05. Because this is an important component of the mother-infant relationship, that may be more prominent at earlier ages, an additional ANOVA was run on the frequency of maternal contacts broken across months 1-3 only. Abusive mothers (*M*=1.81, *SE*=.24) broke more contact with their infants than non-abusive mothers (*M*=1.02, *SE*=.16) , *F*(1, 18)=7.75, *p*<.05. There were no significant group or group by time findings for contacts made between the mother-infant pairs.

Abusive mothers (*M*=.83, *SE*=.22) rejected their infants more frequently than non-abusive mothers (*M*=.19,*SE*=.06), *F*(1, 18)= 8.34, *p*<.05 (see Figure 6). There were no significant group or group by time effects for the duration of grooming bouts between the mother and infant or frequency of maternal restrains.

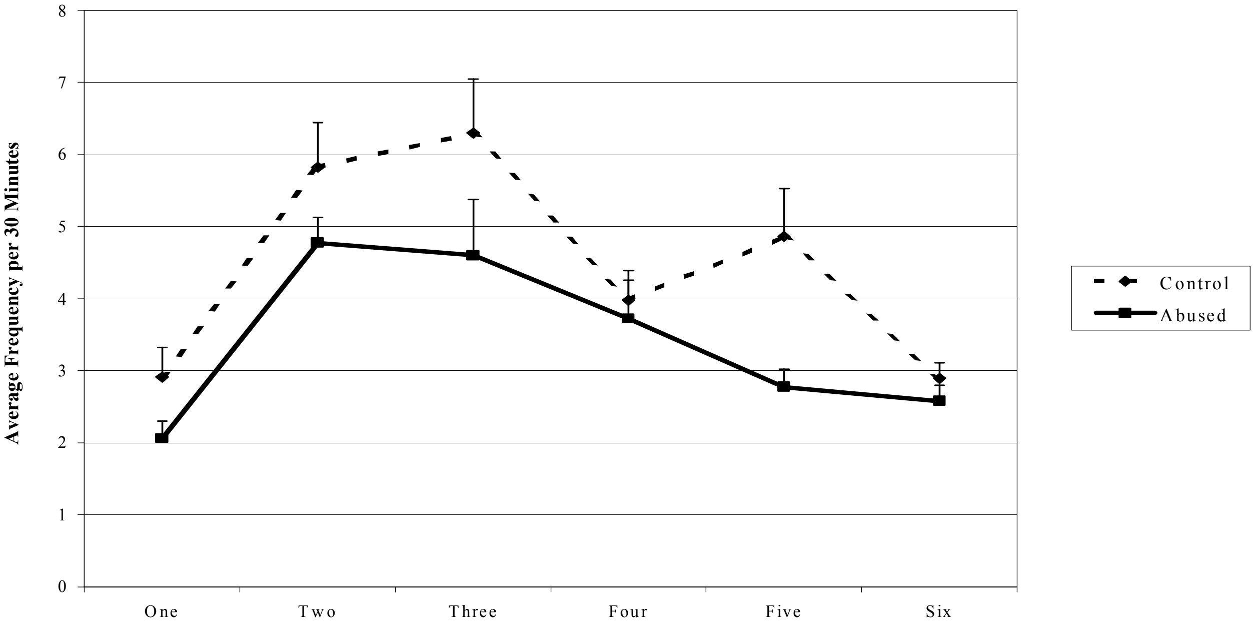
Abused infants (*M*=1.46, *SE*=.54) vocalized more than control infants (*M*=.17, *SE*=.05), *F*(1,18)=5.58, *p*<.05 (see Figure 7). Abused infants (*M*=.56, *SE*=.14) also threw more tantrums compared to control infants (*M*=.15, *SE*=.03), *F*(1,18)=8.15, *p*<.05. There was a significant time by group interaction for proportion of time spent in solitary play, *F*(3.98, 71.71)=2.52, *p*<.05 (see Figure 8). Six Bonferroni corrected t-tests failed to determine where the group by time

Figure 3: Proportion of time spent in other contact between infants and mothers



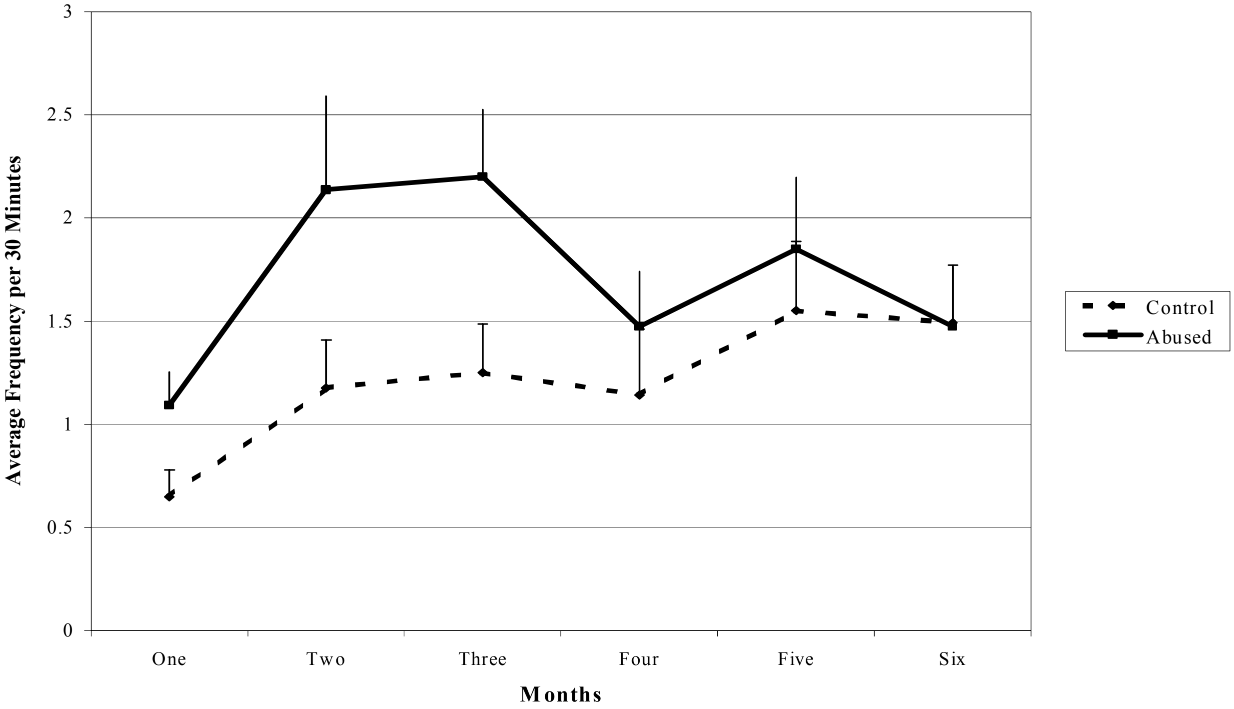
Note: Group x Time effect. Post-hoc tests not significant.

Figure 4: Frequency of contacts broken by infant to mother



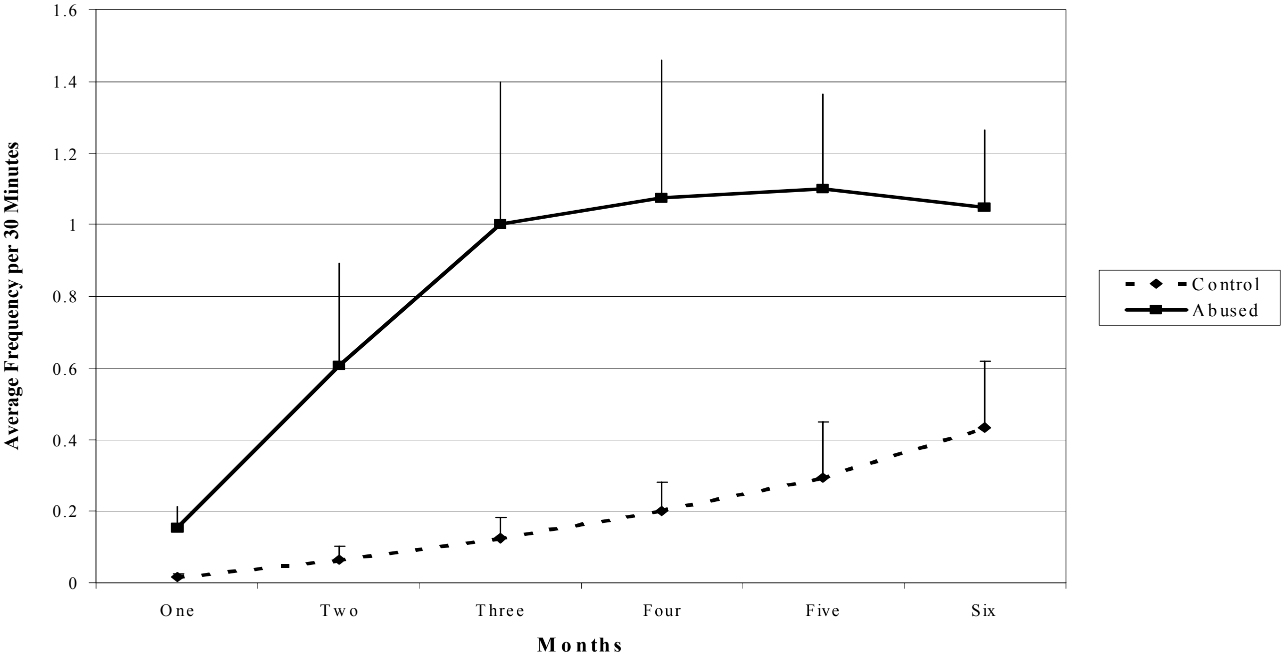
Note: Group main effect. Control infants broke more contact than abused infants.

Figure 5: Frequency of contacts broken by mother to infant



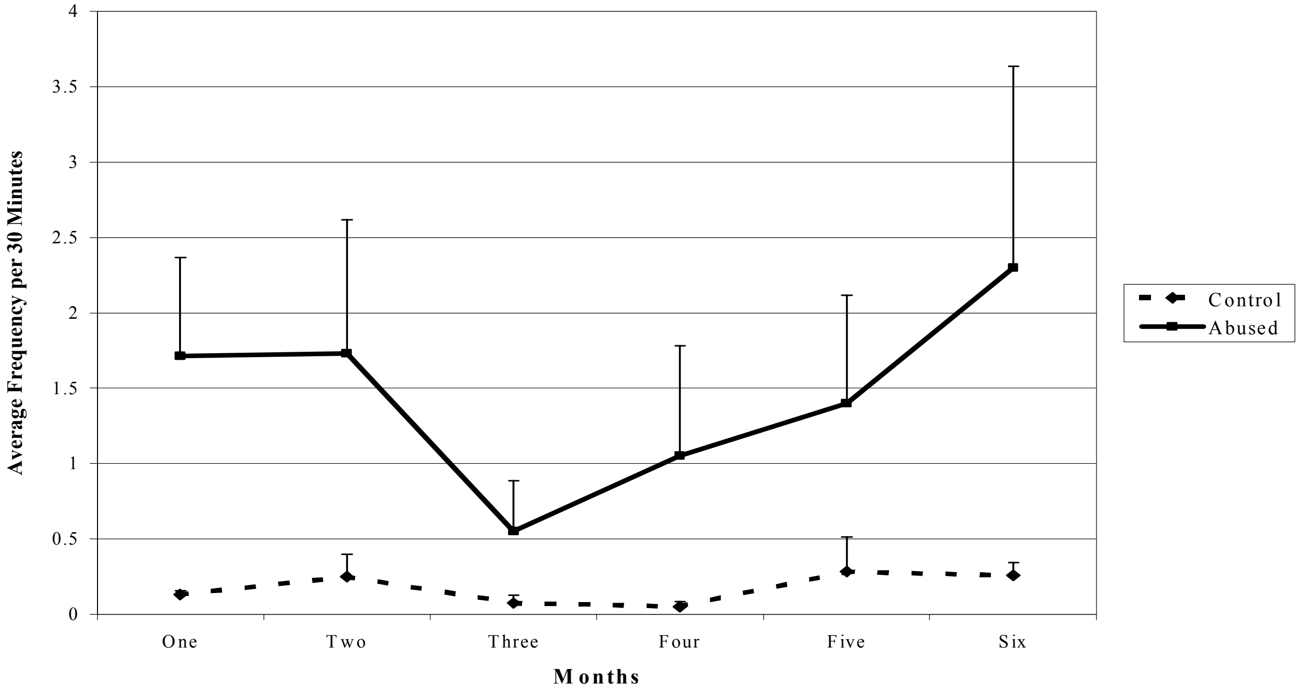
Note: Group main effect. Abusive mothers broke more contact with infants than control mothers.

Figure 6: Frequency of maternal rejections



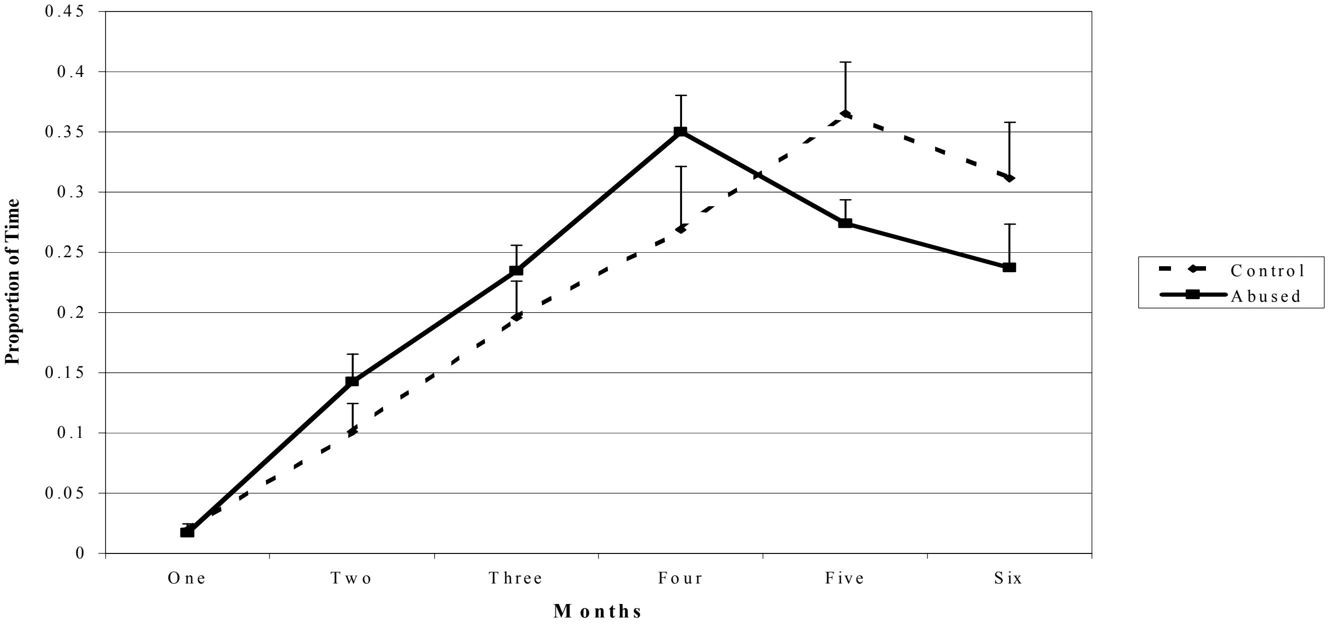
Note: Group main effect. Abusive mothers rejected infants more than control mothers.

Figure 7: Frequency of infant vocalizations



Note: Group main effect. Abused infants vocalized more than control infants.

Figure 8: Proportion of time spent in solitary play



Note: Group x Time effect. Post hoc tests not significant.

interaction was occurring. There were no significant group or time by group effects for infant nervous behavior or for any of the observed interactions between infants and others.

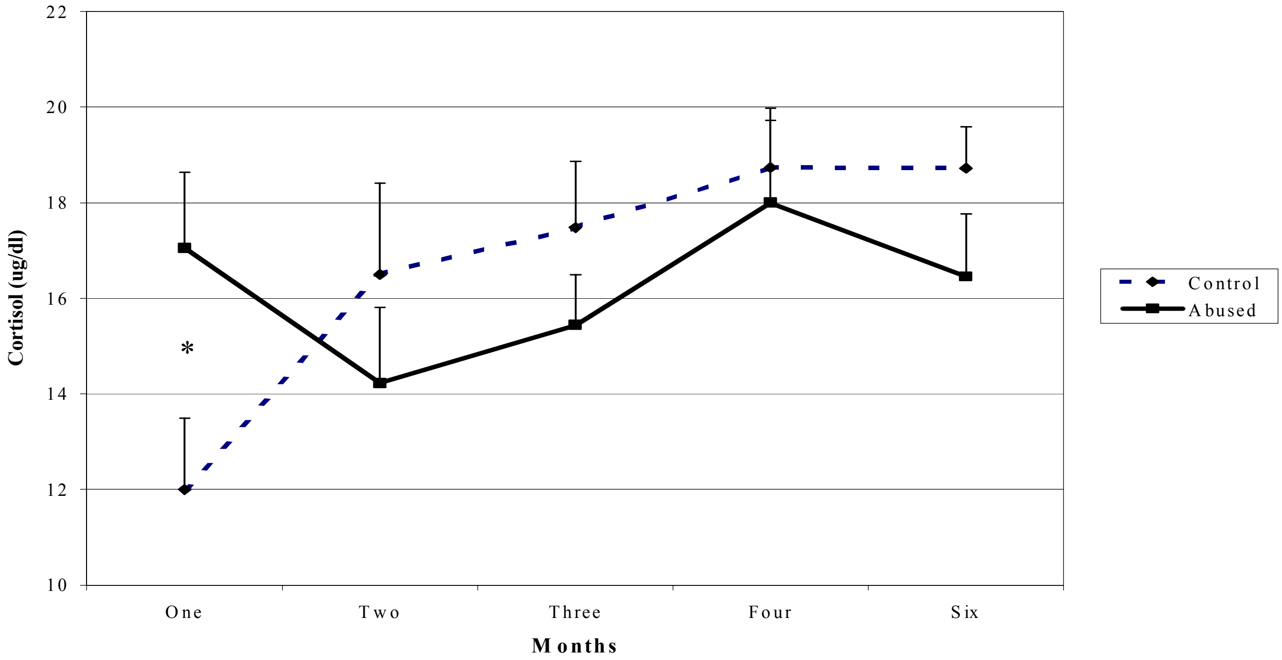
Physiological Data

There was a significant time effect for basal levels of cortisol across the first six months of life (see Figure 9), *F*(4, 7) = 2.44, *p*=.05. Although mean cortisol levels appeared lower at month 1(*M*=14.52, *SE*=1.21) compared to month 6, (*M*=17.58, *SE*=.81), the statistical analysis failed to find a difference, *t*(19) = 1.95, *p*=.07. Although there was not a significant group main effect for basal cortisol levels, there was a significant group by time interaction (see Figure 9), *F*(4, 72)=2.45, *p*=.05. An a priori planned comparison at week 4 revealed that abused infants (*M*=17.05, *SE*=.16) had higher levels of cortisol than control infants (*M*=12.00, *SE*=1.49), *t*(18)=2.32, *p*<.05. After the first month, control infants consistently had higher levels of basal cortisol compared to abused infants (see Figure 9), but parametric tests failed to find differences between the two groups.

There were no group or group by time effects for the month 4 morning and evening measures of cortisol, indicating that the two groups did not differ on their cortisol circadian rhythm. Cortisol levels did decrease from morning to evening, *F*(1, 18) = 43.62, *p*<.01 (see Figure 10). Difference scores between morning and evening samples were not significantly different when the two groups were compared; the degree of cortisol change across the day was similar for the two groups.

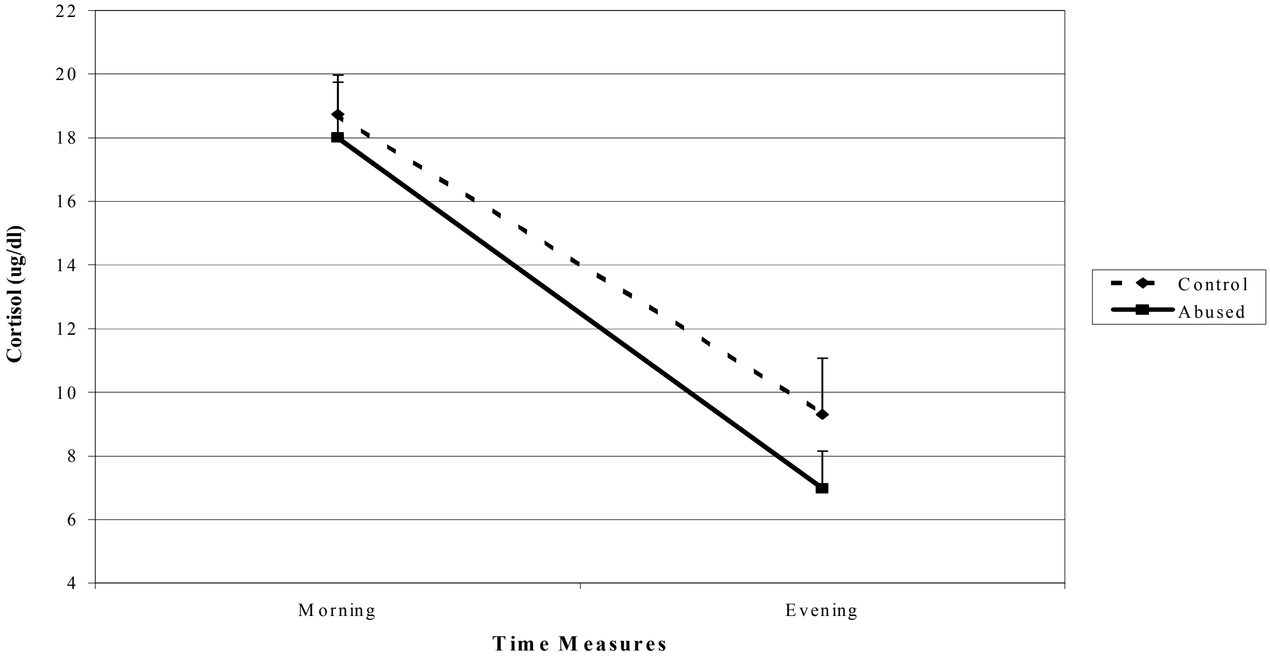
Although it appeared that at month 2 the abused infants mounted a larger cortisol response from baseline sampling to post-maternal buffering sampling (*M*=12.00, *SE*=3.64) compared to control infants (*M*=3.82, *SE*=2.16), the statistical analysis failed to find a difference

Figure 9: Basal cortisol levels across the first six months



Note: Group x Time effect: Abused infants had higher levels of cortisol at month 1.
*$p<.05$

Figure 10: Morning and evening basal cortisol levels



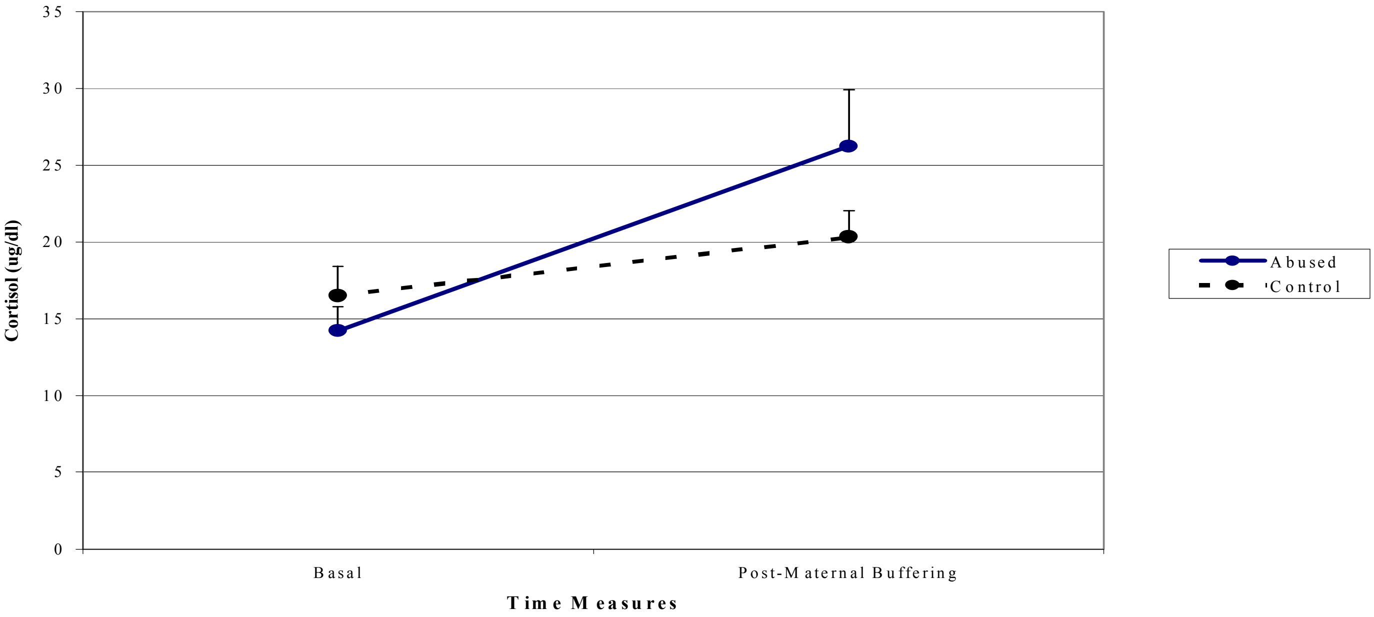
Note: Time effect. Cortisol levels decreased across the day.

t(18)=1.94, *p*=.07 (see Figure 11). There were no group or group by time effects for the separation test conducted at month 3; the two groups did not differ in their cortisol response to stress, or to the two hour reunion. There was a significant effect for time, *F*(2, 17) = 41.30, *p*<.01 (see Figure 12). Infants demonstrated an increase in cortisol from baseline (*M*=16.42, *SE*=.92) to post-30 minute separation (*M*=35.25, *SE*=2.02), *t*(18)=12.06, *p*<.001. Infants also demonstrated a decrease from the post-30 minute separation (*M*=35.25, *SE*=2.02) to the post-2 hour reunion (*M*=25.31, *SE*=1.71), *t*(18)=4.00, *p*<.01. Levels of cortisol after the 2-hour reunion (*M*=25.31, *SE*=1.71) were still significantly higher than baseline levels (*M*=16.42, *SE*=.92), *t*(18)=4.70, *p*<.01, indicating that though the infants cortisol decreased upon reunion with their mothers, it did not return to baseline levels. The independent t-tests that were calculated on the difference scores failed to detect any differences in stress response between the two groups.

There were no group or group by time effects for the separation test conducted at month 6; the two groups did not differ in their cortisol response to separation. Infants did demonstrate a significant increase in cortisol from resting levels (*M*=17.52, *SE*=.79) to post-30 minute separation levels (*M*=31.29, *SE*=1.46), *F*(1, 18) = 152.72, *p*<.01 (see Figure 13). Abused and non-abused infants did not differ in their difference scores of basal and post-30 minute separation cortisol values.

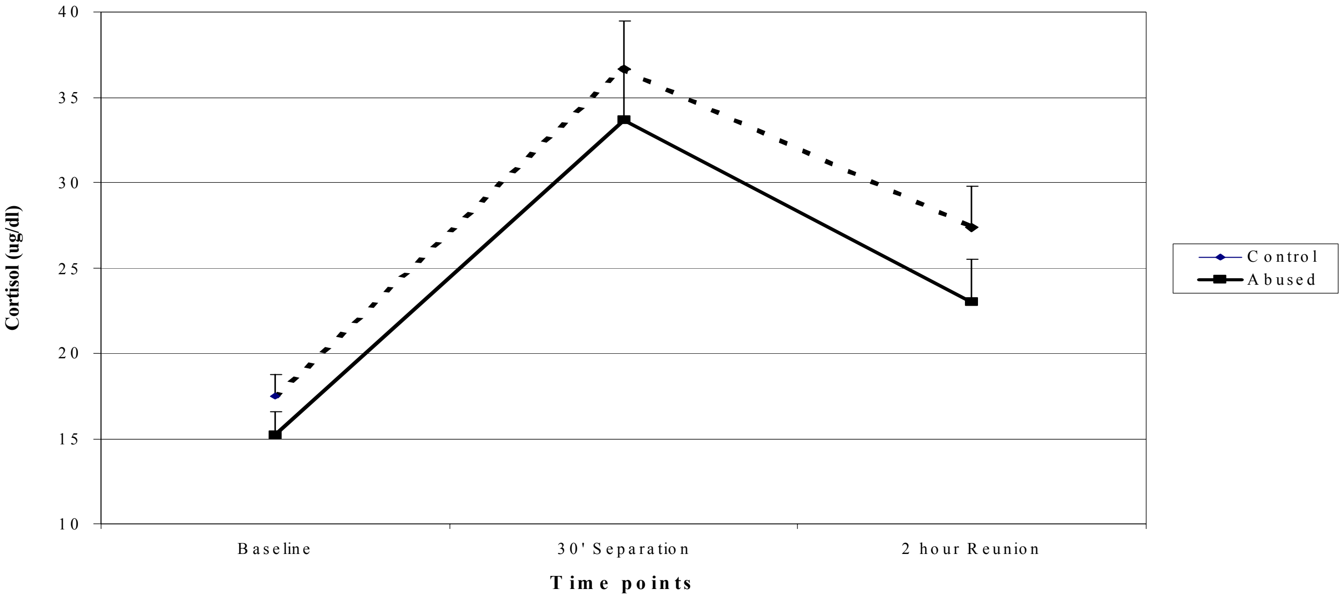
Neither a significant group or group by time effect was found when the difference scores from the month 3 separation test were compared to the difference scores of the month 6 separation test. However, there was a time effect, *F*(1, 17) = 10.13, *p*<.01, with animals exhibiting larger increases in cortisol from baseline to post-separation at month 3 (*M*=18.81, *SE*=1.56) compared to month 6 (*M*=13.92, *SE*=1.14), see Figure 14.

Figure 11: Cortisol response at month 2 maternal buffering test



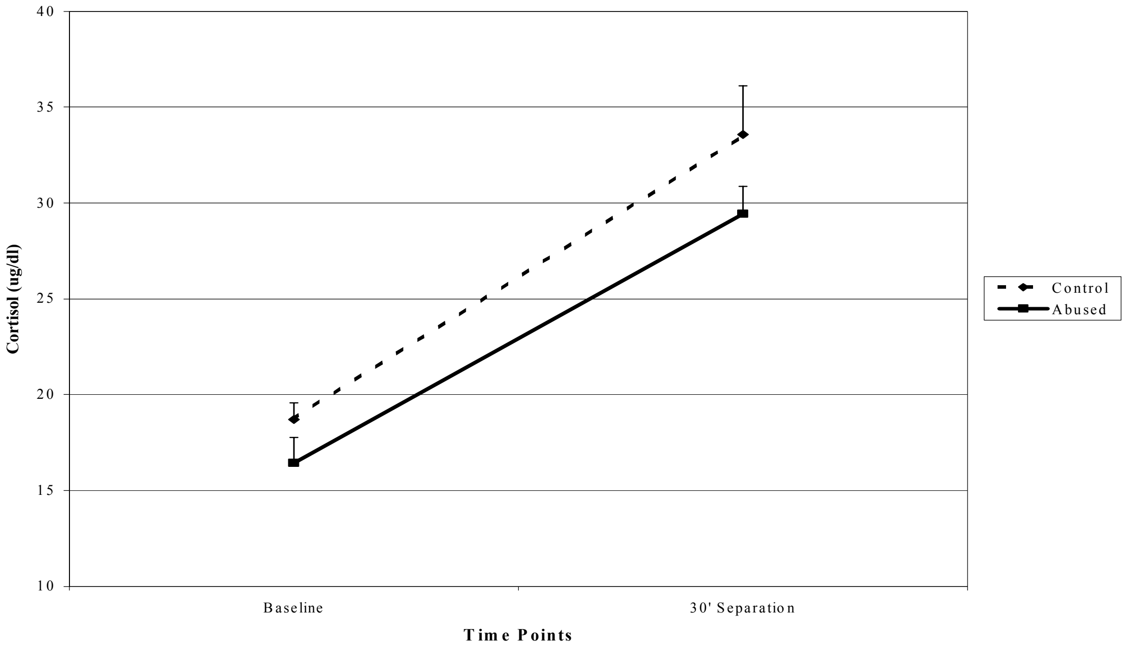
Note: Difference scores marginally significant between abused and non-abused infants.

Figure 12: Cortisol response across month 3 separation test



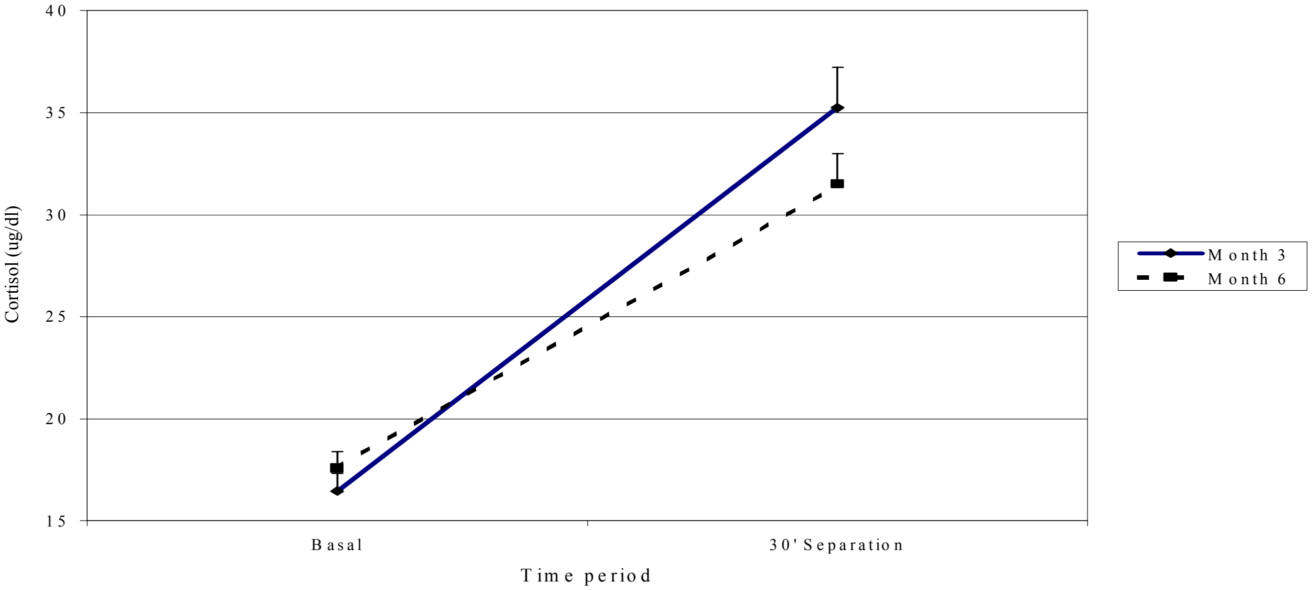
Note: Time main effect. Cortisol increased from T1 to T2, and decreased from T2 to T3.

Figure 13: Cortisol response at month 6 separation test



Note: Time main effect. Cortisol increase was larger at Month 3 than Month 6.

Figure 14: Cortisol response to stress across months 3 and 6



Note: Time main effect. Cortisol increased from baseline to post-separation

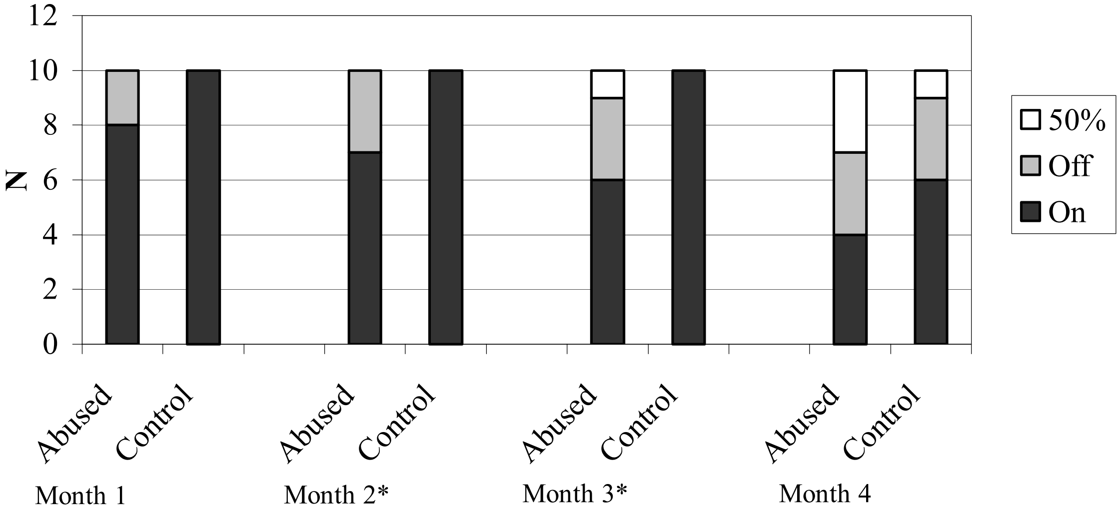
Although there was only one significant time point in which the abused and non-abused animals differed in their levels of cortisol (month 1), across all ten measures of cortisol activity (month 1 basal, month 2 basal, month 2 post-maternal buffering, month 3 basal, month 3 post-separation, month 3 post-reunion, month 4 morning basal, month 4 evening basal, month 6 basal, month 6 post-stress), a sign test revealed that abused infants demonstrated lower levels of cortisol when compared to the control infants eight out of ten times, $p<.05$.

Mother and Infant Monthly Assessment Data

Compared to the non-abused infants, abused infants were less likely to be on their mothers when in the capture unit at the month 2 assessment $\gamma = 1.0$, $p<.05$, and at the month 3 assessment, $\gamma=1.0$, $p=.01$ (see Figure 15). Compared to non-abusive mothers. abusive mothers were also less likely to be holding their infants in the squeeze cage, at month 1, $\gamma = 1.0$, $p=.01$, at month 2, $\gamma = .77$, $p <.05$, and at month 3, $\gamma = 1.0$, $p=.01$ (see Figure 16). Compared to control mothers, abusive mothers were less distressed at the researcher's attempt to remove the infant from her at month 2, $\gamma = -.85$, $p<.001$, at month 3, $\gamma = -.75$, $p=.01$, and at month 4, $\gamma = -.85$, $p <.01$ (see Figure 17). Abusive mothers also vocalized less to their infants during the procedures compared to control mothers, at month 3, $\gamma = -1.0$, $p<.05$, and at month 4, $\gamma = -1.0$, $p <.05$ (see Figure 18).

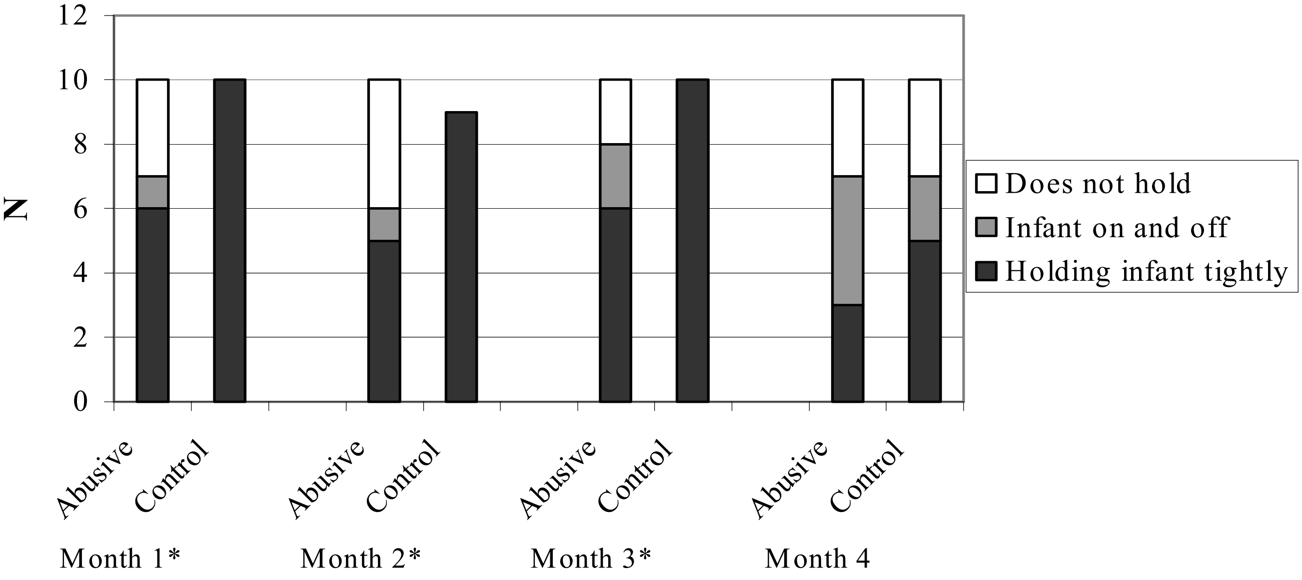
Compared to control infants, abused infants were less likely to resist during the blood draw procedure at month 2, $\gamma = -.913$, $p <.001$ (see Figure 19). Abused infants were also less likely to show tantrums and body jerks during the procedures at month 2, $\gamma = -.78$, $p <.01$, and at month 3, $\gamma = -.64$, $p <.05$, compared to control infants (see Figure 20). Compared to abused infants, control infants were more difficult to console during procedures at month 2, $\gamma = -.66$,

Figure 15: Infant use of mother in the capture unit



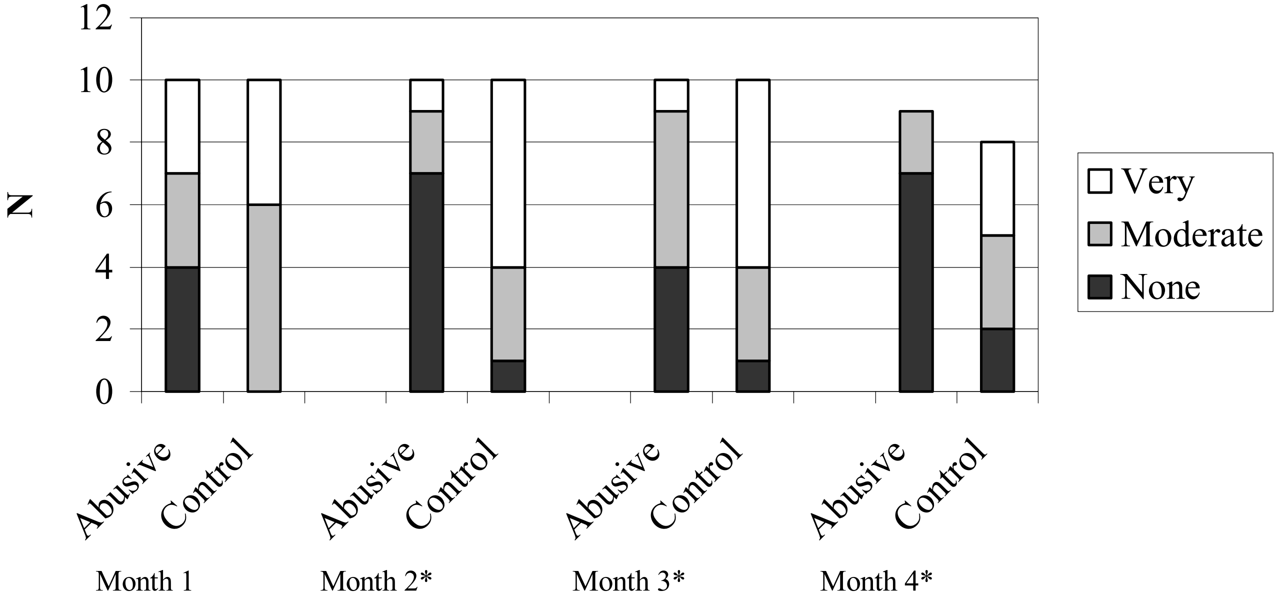
Note: Abused infants were more likely to be off of their mother at Months 2 and 3.
**p*<.05

Figure 16: State of mother in squeeze cage



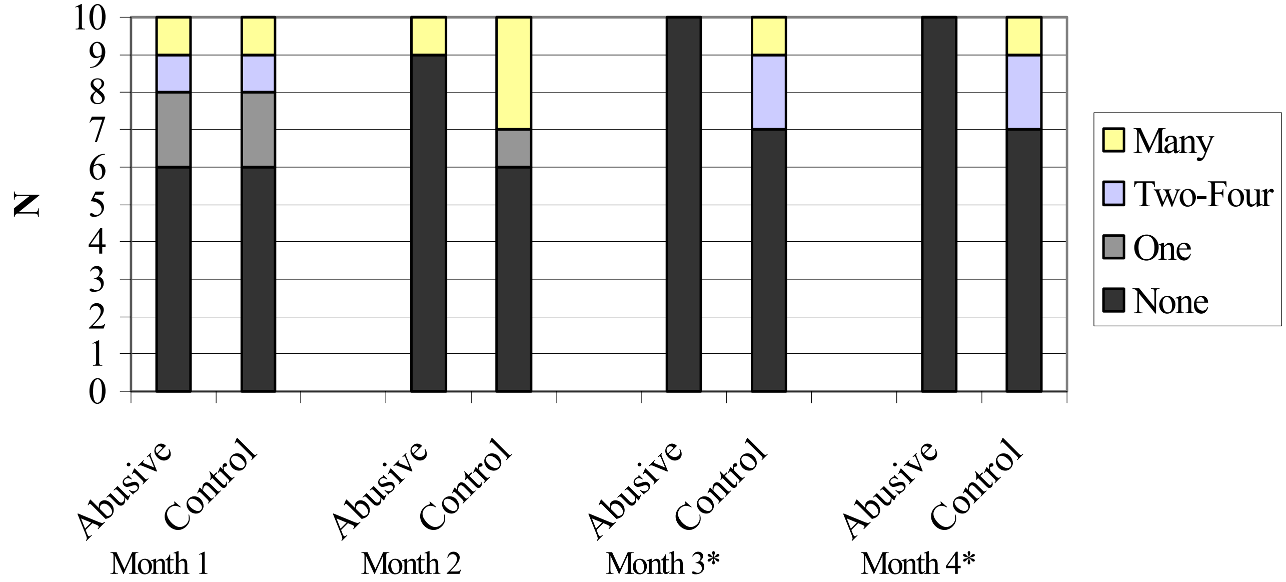
Note: Abusive mothers were more likely to not hold infant at Months 1, 2 and 3.
**p*<.05

Figure 17: Maternal distress at infant removal



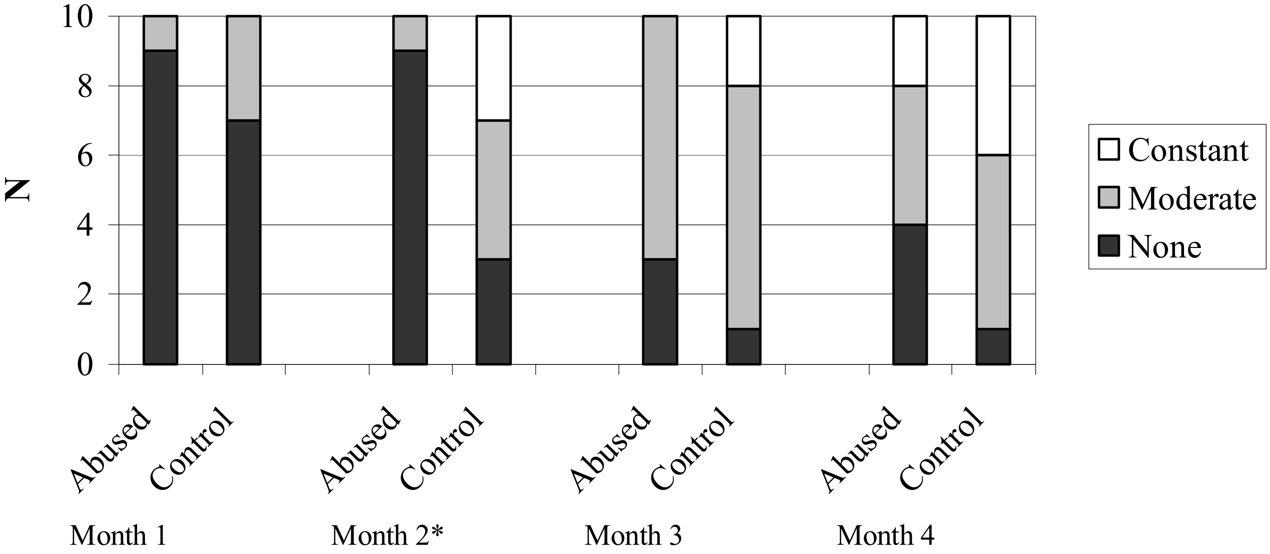
Note: Abusive mothers less distressed at infant removal at Month 2, 3, and 4.
**p*<.05

Figure 18: Maternal vocalizations to infant



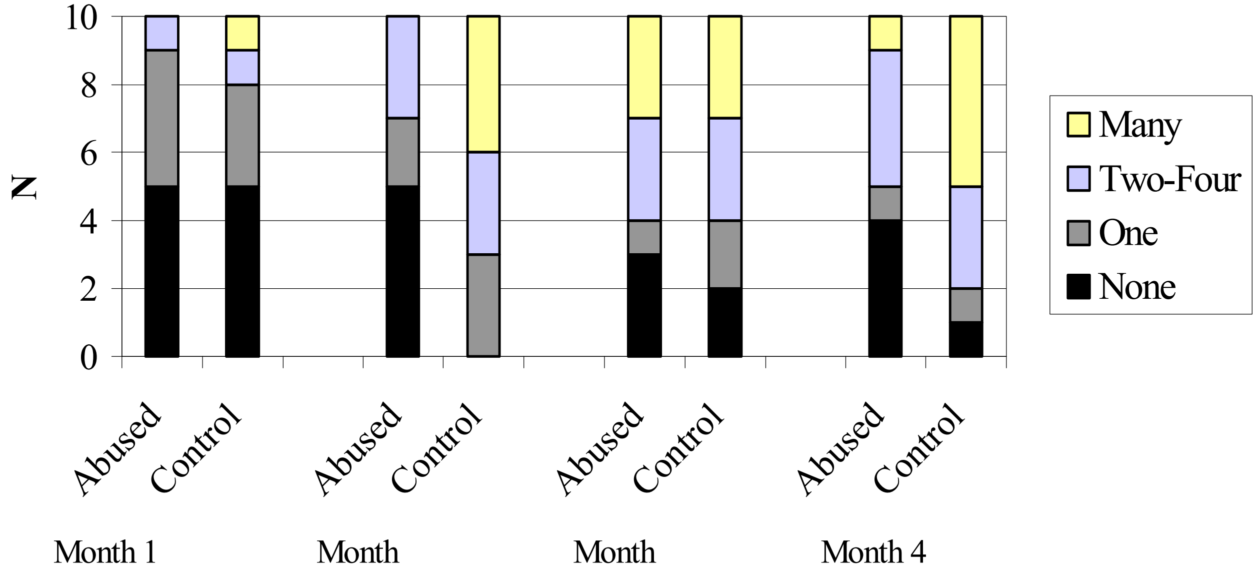
Note: Abusive mothers vocalized less to infants at Month 3, and 4.
**p*<.05

Figure 19: Infant resistance to blood draw and handling



Note: Abused infants less resistant to handling at Months 2.
p<.05

Figure 20: Frequency of infant tantrums and body jerks during handling



Note: Abused infants had fewer tantrums and body-jerks at Month 2 and 3.
p<.05

p<.05, and they vocalized more during procedures at month 2, γ= -.64, *p*<.05. No other differences were found between groups on the remaining assessment items.

2-hour Reunion (post-separation) Data

Independent t-tests failed to find differences between the two groups on any of the frequencies or durations outlined in Table 4.

Attachment Data

The data were evaluated to determine if abused infants demonstrated qualitatively different "attachment styles" from the non-abused infants. Abused infants broke contact with their mothers less often than did non-abused infants *F*(1, 18)=14.72, *p*<.01, and abused infants were less likely to be on their mothers during the blood sampling periods at the month 2 assessment γ= 1.0, *p*<.05, and at the month 3 assessment, γ=1.0, *p*=.01. There was also a significant group by time interaction for the proportion of time spent in contact between infants and their mothers *F*(4.33, 77.91)=2.46, *p*<.05. Bonferroni corrected post-hoc tests failed to determine where this interaction was occurring. There were no differences between abused and non-abused infants in the frequency of infant initiated contacts in the home environment. There were no differences in proportion of time spent in social play with others. Likewise, there were no differences between abused and non-abused infants in the duration of time spent on their mothers, or in the duration of time spent exploring during the last 30 minutes of the two-hour reunion.

CHAPTER 4

DISCUSSION

The results of this study provide partial support for several of the original hypotheses. As predicted, abused infants broke contact with their mothers less often than control infants, and abusive mothers broke contact with their infants more often during the first three months compared to control mothers. However, perhaps because abusive mothers were breaking contact more often with their infants, support for the hypothesis that abused infant would spend a greater proportion of time on their mothers was not obtained. The quality of contact differed between abused and non-abused infants. There was a group by time interaction for proportion of time spent in ventral contact and other contact. The data suggests that control infants may have spent more time in ventral contact than abused infants during the first four months, and that abused infants spent more time in other contact during the first two months. This is congruent with the hypothesis that the quality of contact would be lower in the abused infants compared to the control infants. Abusive mothers demonstrated higher rates of rejections towards their infants during the six months, compared to control mothers. This is supportive of the hypothesis that abusive mothers would demonstrate different maternal styles compared to non-abusive mothers.

As predicted, abused infants also demonstrated higher rates of vocalizations and tantrums during the first six months compared to control infants. This may be due to the abuse and/or the lower quality of maternal care they were receiving. Differences were also found in the proportion of time spent in solitary play. Post-hoc tests were unable to detect where the group by time interaction was occurring, although an examination of Figure 8 suggests that abused infants engaged in equivalent, or slightly more solitary play during the first four months of life compared to control infants, and that they engaged in less solitary play during months 5 and 6. Abused infants did not differ from non-abused infants on any measure of interactions with others. Abused infants also did not differ from non-abused infants in rates of nervous behavior during the six months. Thus, support was not obtained for any of the original hypotheses that focused on infant behavior when off of the mother. It appears that, although the mother-infant relationship may be different in abused and non-abused mother-infant pairs, infants may develop normal social interactions during the first six months.

As predicted, abused infants had higher levels of basal cortisol during the first month of life compared to control infants. This also corresponded with the period of maximum abuse. A sign test indicated that abused infants consistently demonstrated lower levels of cortisol (basal and in response to separation) after month 1 compared to non-abused infants. This lends support to the argument that the HPA axis of the abused infants began exhibit hypocortisolism after month 1. As discussed by Gunnar and Vazquez (1998) the adverse experiences of the abused infants may have caused increased levels of cortisol that, subsequently may have resulted in the downregulation of components of the HPA axis. This would result in lower rather than higher cortisol secretion later in life. This hypocortisolism would reflect the organism trying to maintain stability by creating the opposite effect that brought about the change, which has been termed “allostasis” (McEwen, 1998).

The changes in cortisol levels for the two groups during the month 2 maternal buffering test, though not significant, were suggestive. After month 1, this was the only time point in which the abused infants demonstrate higher levels of cortisol compared to the control infants. Under normal conditions, mothers typically buffer the stress response of their infants during this type of test (Levine et al., 1985). Figure 11 demonstrates that there was very little change in cortisol for the control infants, but that there was an increase in cortisol for the abused infants. The data suggest that abusive mothers may not have been buffering the stress response of their infants, while the control mothers did.

The hypothesis that abused infants would exhibit different circadian activity compared to control infants was not supported. It may have been the case that the month 4 measurement point was too early to detect differences in circadian activity. The change in cortisol levels from baseline to post-separation was equivalent for the abused and non-abused infants at month 3 and 6. Perhaps separation was too severe of a stressor to elicit differences in mean cortisol changes between the groups.

Many of the significant results found in the mother-infant assessments occurred during the month 2 and 3 assessments. During the process of being captured, abused infants were less likely to be in physical contact with their mothers compared to non-abused infants. During the procedures the abusive mothers did not produce as many vocalizations or signs of distress at infant removal as the non-abusive mothers did. The abused infants were less responsive to being handled; they did not demonstrate as many tantrums or body jerks as the control infants, and they were easier to console by researchers. This was consistent with the hypothesis that if there were differences in HPA reactivity, there would be differences in behavioral reactivity. In comparison to non-abusive mothers, abusive mothers appeared to be less protective of their infants, and they contacted their infants less. This would suggest that the decreased levels of reactivity observed in the abused infants' during times of capture may have been a coping mechanism. It would have been adaptive for the abused infants to not struggle during times of stress, and to not draw attention to themselves; they may have learned that their mothers would not rescue or protect them. Control infants, on the other hand, had experienced responsive mothers, who held them during stressful times, and who called to them when they were separated. It would have been to their advantage to struggle during blood draws, and attempt to get back to their mothers, who were vocalizing to them.

Contrary to prediction, there were no differences in how abused and non-abused infant responded during the last 30 minutes of the post-separation reunion period at month 3. There was very little variability in the proportion of time that infants spent off of their mothers during the last 30 minutes of taping. Very few infants even broke contact with their mothers, and those that did explored very little. It may have been the case that the procedure itself was too stressful for infants to exhibit any kind of exploratory behavior. Perhaps if the mother-infant pairs had been reunited for longer than 2 hours, infants would have been more likely to explore. In the present paradigm it appears that the infants' system was stressed so much that individual variation in behavior was not observable.

I had predicted that there would be differences in attachment quality among the abused and non-abused infants. Abused infants did display lower rates of breaking contact with their mothers, as would be expected of insecurely attached infants. Compared to non-abused infants, abused infants were less likely to be on their mothers when researchers ran the mother-infant pairs into the building, which could be indicative of an insecure attachment (specifically, insecure-avoidant). Although there was a group by time interaction for proportion of time mothers and infants spent in contact, post-hoc tests determined that there were no differences between abused and non-abused infants at each monthly time point. Abused infants did not differ from non-abused infants in the proportion of time they spent interacting with other animals. There were also no behavioral differences between abused and non-abused infants during the last 30 minutes of the post-separation reunion. Thus, it remains unclear as to whether there were differences in the quality of attachment in abused and non-abused infants. There were clear differences between abusive and non-abusive mother-infant pairs in contact frequency and quality, which is a component of attachment. However, because there were no differences in how the infants interacted with others, it does not appear that the mother-infant relationship impacted how abused infants interacted with their environment. Because this is such a crucial aspect of attachment theory, it cannot be concluded that abused infants were insecurely attached or that non-abused infants were securely attached.

The data suggest that abusive and non-abusive mother-infant pairs have a different relationship with one another, as demonstrated by the differences in contact quality (ventral and other contact), and the differences in contacts made and broken by mothers and infants. Abuse, and/or the overall difference in mother-infant interactions among the abusive mother-infant pairs (compared to controls), was also associated with infants who were less likely to remain in contact with their mothers during stressful times, and who were less reactive and responsive to being handled. This corresponds with the cortisol data, in which after month 1, abused infants consistently exhibited lower cortisol levels than control infants. Not only were abused infants less behaviorally reactive during the stressful procedures compared to control infants, their cortisol levels were lower across the six months except at month 2 when the abusive mothers may not have buffered their infants' stress response. This suggests that early adversity (abuse or altered mother-infant interactions) may lead to animals that have difficulty regulating their stress response in the presence of their mothers at a young age, and that have blunted behavioral and physiological responses.

Several of the findings in this study were consistent with the results of previous studies. Similar to Coplan et al. (1996), who found that early adverse experiences (variable foraging demands) affected mother-infant contact duration and quality, this study also found alterations in these variables in the abused infants. Alterations in mother-infant quality in abused mother-infant pairs have also been reported by Maestripieri (1998). This suggests that in addition to being abusive, abusive mothers generally treat their infants differently than do non-abusive mothers.

Similar to handled rat pups, the abused infants in this study demonstrated a blunted HPA axis response. Abused infants were more likely to have lower basal and post-stressor levels of cortisol compared to non-abused infants. Several researchers, who examined basal and post-separation levels of cortisol in mother-reared and peer-reared animals found that peer-reared animals had lower resting levels of cortisol, and a smaller response to stress (Clarke et al., 1998; Shannon et al., 1998). Dettling and colleagues also reported hypofunctionality of the HPA axis, with lower basal morning levels of cortisol in marmosets that had experienced repeated maternal separations (Dettling, Feldon & Pyrce, 2002).

Lower resting levels of cortisol were also reported in adult survivors of child maltreatment (Bruce et al, 2000; Cicchetti & Rogosch, 2001; Gunnar & Vazquez, 2001; Heim et al., 2001). The pattern suggested in previous research, and in this project, is one in which the system excretes high levels of cortisol during the period of adversity, and then begins to produce lower levels of cortisol after this period. This is similar to one pattern of cortisol activity and reactivity observed in PTSD patients, but should not be interpreted as the only symptom of PTSD (Heim et al., 2000).

Unlike previous studies conducted with human and non-human primates, Alessandri, 1991; Dodge et al., 1990; Fagot et al., 1989; Haskett & Kistner, 1991; Ruppenthal et al., 1976) this study failed to show that early adverse rearing experiences caused infants to interact with others (non-mother) differently. It may be that maternal abuse was not as severe an experience for these animals as peer-rearing, repeated separations, and the types of maternal maltreatment experienced in humans. Because there were no differences found in how the abused and non-abused interacted with other animals, I was not able to conclude that the abused infants demonstrated insecure attachments or that non-abused infants demonstrated secure attachments. It may also be the case that while there were alterations in the mother-infant relationship, the infants were resilient enough to develop normal social interactions with others. If this was in fact occurring, then while the abused infants were able to interact with animals similarly to the non-abused animals, it appears that, in the background of this, their HPA axis was responding differently, with lower levels of cortisol. This would be further evidence to suggest that behavior outcomes do not always correspond with physiological outcomes as demonstrated by Levine and his colleagues (1984, 1987, 1988).

One shortcoming of this study was the large variability in abusive episodes in the abusive mother-infant pairs. Hourly rates of abuse over the three months ranged from .30 to 6.4, with an average rate of 1.65 abusive episodes per animal. It is possible that a small number of abusive episodes do not drastically alter the behavioral and physiological development of an individual. In the human literature, subjects are studied with much higher episodes of reported abuse than those observed in this study. Thus, the frequency of abusive episodes should be considered when looking for alterations in behavior and HPA axis function.

Abuse alone may not be what caused the differences observed between abused and non-abused infants. The abusive mothers also exhibited alterations in how they responded and cared for their infants. It is certainly the case that there are variations in maternal care in the non-abusive mothers. Thus, instead of focusing on the effects of maternal abuse, as defined in this paper, it may be more constructive to compare the effects on development of infants who receive high quality maternal care with infants that receive low quality maternal care. Measures that could be used to classify animals into these groups would be abuse, rejections, contacts made and broken, contact duration, and quality of contact. These were all variables in which differences were found between the abused and non-abused animals, although there was individual variability in these variables in the non-abusive mothers. Human mothers can exhibit poor maternal care (neglect), without being abusive, and cause similar alterations in behavioral and physiological development of their children. By broadening the definition of what constitutes maladaptive maternal styles for infants, we may gain a better a picture of what can ultimately affect development.

The assessment scales that were used to evaluate the mothers and infants at each month yielded several promising results. These were questions that were adapted from a larger temperament scale created by Schneider and Suomi (1992). Using similar infant assessment questions, M. Champoux (personal communication, July, 2003) compared the reactivity of nursery-reared and mother-reared infants. She too found lower reactivity among the nursery-reared infants. Thus, these assessments appear to be detecting differences in how infants from adverse rearing conditions are reacting during times of stress, specifically during months 2 and 3. These scales should be used in conjunction with attachment measures to help detect behavioral differences among infants.

This study illustrates that not only were levels of cortisol consistently lower in abused infants compared to non-abused infants, but that over the six months of development basal cortisol may increase with time. It would be of interest to continue to examine the behavioral and physiological development of these infants for several years, to determine if cortisol levels continue to increase, and at what point they level out. Puberty marks a drastic change in multiple physiological systems of these animals, so it would be important to examine the interaction of puberty and early adversity on the HPA axis.

Finally, this study only focused on cortisol activity in these animals. This of course is only one aspect of the HPA axis, specifically the adrenal output. Alterations in cortisol activity among groups of animals indicate alterations in adrenal function, but not the cause. Lowered cortisol in abused animals could be due to any one of the following: decreased levels of CRF, decreased levels of ACTH, decreased CRF receptors on the pituitary, decreased ACTH receptors on the adrenal gland, or decreased cortisol receptors in the negative feedback loop. Measurement of ACTH levels would help determine whether it is just the adrenal gland that is malfunctioning, or if it is both the pituitary and adrenal that are malfunctioning. Pharmacological tests, such as dexamethasone, ACTH, and CRF challenges, would also help clarify where HPA axis abnormalities exist.

This study has examined the behavioral and HPA axis development (as measured by cortisol) during the first six months of life in maternally abused and non-abused rhesus macaque infants. Abused infants demonstrated alterations in their interactions with their mothers, had larger increases in cortisol during a maternal buffering test, and had consistently lower basal and stress response levels of cortisol after month 1 compared to controls. This was consistent with the assessments that were made of the infants at each month, which suggested that they were less reactive and responsive to being handled. This suggests that the behavioral and physiological systems of the abused infants may have begun to downregulate in response to early adversity, thereby exhibiting blunted behavioral and cortisol responses. The next task is to determine how the systems of these infants continue to develop, and whether this development is similar to that of maltreated humans. Of interest will be whether these animals exhibit other symptoms similar to those in patients with PTSD and/or depression. This could ultimately shed light on the developmental consequences of abuse, as well as clarify potential precursors to psychological disorders.

APPENDIX A

LITERATURE REVIEW OF HUMAN STUDIES

Most studies that examine stress in children focus on the effects of maltreatment, though several have examined the outcomes of children raised in orphanages. Of these, most examine behavioral outcomes, with only a few examining the effects on the HPA axis. One of the many difficulties in examining maltreatment in children is that it is almost impossible to separate those children that have been physically abused, from those that have been sexually abused, from those that have been neglected. It is also difficult to determine if the observed effects are due to maltreatment solely, or if they may be due to malnutrition, inadequate medical care, inadequate stimulation, or any host of variables that typically co-occur with early developmental stress. Finally, many of these studies are retrospective and/or correlational. Thus, it is important to be aware of these difficulties and confounds when reviewing the literature discussed below.

Many researchers have examined the behavioral outcomes of maltreated children. Alessandri (1991) found that children who had a history of physical abuse, sexual abuse, or neglect, engaged in less play, and engaged in more aggression than the control group. The abused children were also more likely to be rated by their teachers as more emotionally disturbed. Fagot, Hagan, Youngblade, and Potter (1989) also found that physically abused children were more antisocial, disruptive and aggressive than non-abused children. The physically abused youngsters had higher rates of negative activity (physical and verbal aggression) compared to non-abused children (see also Dodge, Bates, & Pettit, 1990; George & Main, 1979; Kaufman & Cicchetti, 1989 for similar results). Other researchers have reported that young physically abused children initiated fewer positive interactions with their peers, displayed avoidance behavior, and had a higher number of negative interactions (Haskett & Kistner, 1991; Jacobson & Straker, 1982).

Maltreated children are also more likely to have conduct disorders and behavior problems (Hart, Gunnar, & Cicchetti, 1995). Several studies have indicated that early forms of childhood stress predisposed children to mood and anxiety disorders, specifically depression and PTSD (Bremner, Southwick, Johnson, Yehuda, & Charney, 1993; Mullen, Martin, Anderson, Romans, & Herbison, 1996; Stein et al., 1996). Sexually abused children demonstrated the worst symptoms, with high rates of major depression, suicide attempts, self-mutilation, somatic complaints, poor self-esteem, anxiety disorders, sleep disturbances, substance-abuse disorders, learning disabilities, and aggression (DeBellis et al. 1994, Garnefski & Diekstra, 1997; Weiss, Longhurst, & Mazure, 1999).

Several investigators have also examined the effects of orphanage rearing on the developing child, with the assumption that the child has experienced a neglectful and stressful environment (see Johnson, 2000 for a review of orphanage conditions). Results do vary, depending on the state of the orphanage being evaluated. Children raised in Romanian orphanages prior to 1990 demonstrated severe behavioral and emotional problems, with aggressive and antisocial behavior (Johnson, 2000). These children demonstrated delays in emotional, motor, social speech and physical development, as well as impairments in play and exploratory behavior (reviewed in Johnson, 2000). These children also had cognitive deficits, and exhibited lower IQ scores compared to non-orphanage-reared children (Ames, 1997; Carlson et al., 1995; Rutter, 1998). Thus, the behavioral effects of a deprived environment are quite similar to those of an abusive environment on the developing child.

Physiological examination of maltreated and orphaned children yield a more complex picture. Studies on maltreated children provide evidence for both elevated and suppressed activity of the HPA axis. Reports on HPA activity must be interpreted with caution because baseline levels of cortisol and ACTH vary depending on the time of day they were obtained, the age of the participants across studies varies considerably, and because many maltreated individuals may also have a comorbid diagnosis of depression or PTSD. Also, HPA measures may have been obtained via plasma or saliva, making comparisons difficult. There are several studies that report no differences in resting levels of cortisol and ACTH between abused and non-abused children (DeBellis et al, 1994; Kaufman et al., 1997; Lemieux & Coe, 1995). However, more recent studies have reported that resting levels of cortisol and ACTH were lower in children and adults that had experienced abuse and/or neglect, without a secondary diagnosis of PTSD (Bruce, Kroupina, Parker, & Gunnar, 2000; Cicchetti & Rogosch, 2001; Gunnar & Vazquez, 2001; Heim, Newport, Bonsall, Miller, & Nemeroff, 2001). When maltreated individuals also had a secondary diagnosis of PTSD, resting levels of cortisol and ACTH were reported to be higher than controls (Lemieux & Coe, 1995; DeBellis et al. 1994). Romanian orphans had higher levels of cortisol throughout the day, compared to controls, even when tested several years after removal from the orphanage (Gunnar, Morison, Chisolm, & Schuder, 2000). Clearly, the effects of childhood trauma on the HPA axis are quite discrepant, making generalizations difficult.

Children from adverse backgrounds also exhibit deviations in the way they respond to stressors. Megan Gunnar and her colleagues reported that deprivation in infancy and early childhood is associated with elevated levels of cortisol in response to stressors many years later (Gunnar et al., 2000). Heim et al. (2000) found that women who had been physically or sexually abused as children had higher levels of ACTH and cortisol in response to a stress test compared to controls, and that this effect was magnified when the women had a diagnosis of depression. However, Hart et al. (1995) found that preschoolers who had been physically abused and/or neglected had lower levels of cortisol reactivity. On high-conflict days within the classroom, maltreated children did not demonstrate an increase in cortisol at all, compared to the control children, as you would expect. Thus, how a victim of maltreatment responds to a stressor may vary depending on the type and timing of the abuse or neglect, as well as the victim's age at the time of testing.

The HPA circadian rhythm across these individuals also deviates from non-abused children. In many studies, abused children exhibited lower cortisol levels in the morning compared to controls, and the decline across the day to evening levels was not as great as that of non-abused children (Cicchetti & Rogosch, 2001; Hart et al., 1995; Kaufman, 1991). This suggests that the pattern of basal cortisol secretion across the day is flattened (Gilles, Berntson, Zipf, & Gunnar, 2000; Hart et al., 1995). Alternatively, children that have been both physically and sexually abused tended to have much higher levels of cortisol in the morning than non-abused children, and also demonstrated lower levels in the evening (Cicchetti & Rogosch, 2001; Putnam et al., 1991).

Finally, pharmacological tests have been conducted to determine which components of the HPA axis may be functioning abnormally. Sexually abused women who received dexamethasone suppressed their production of cortisol more than non-abused women (Stein, Yehuda, Koverola, & Hanna, 1997). This suggests that these women had an enhanced negative feedback system, such that their HPA response shut down faster than non-abused women, potentially at the level of the pituitary gland or the hypothalamus. Other researchers have examined the effects of an injection of CRF on the production of ACTH and cortisol. Sexually abused children produced less ACTH in response to CRF administration, and similar levels of cortisol after the injection compared to controls (DeBellis et al., 1994). Heim et al. (2001) also found similar results in depressed women who were sexually abused as children. However, in sexually abused women without depression, the ACTH response to CRF was greater than in non-abused women (Heim et al., 2001). Likewise, Kaufman et al. (1997) found that abused children with depression exhibited higher levels of ACTH post-CRF than control children, yet similar levels of cortisol. These results indicate a potential irregularity at the level of the pituitary gland, such that there is an increased or decreased secretion of ACTH in response to CRF. Unfortunately, pharmacological studies in this area are relatively new, making it very difficult to draw clear conclusions about where in the HPA axis abnormalities exist.

APPENDIX B

LITERATURE REVIEW OF RODENT STUDIES

Rats have been the most widely used subjects for the investigation of the effects of stress on the neuroendocrine system. The developmental period of rat pups is relatively short, yielding information about the developmental consequences of early stress in a short period. The physiology of the rat's HPA axis undergoes rapid development in the first several weeks after birth (Hennessy, 1996). From the age of post-natal day (PND) 4 to 14, the HPA axis undergoes a period of hyporesponsivity, in which basal CORT and ACTH levels are low. When exposed to certain stressors, the response of the HPA axis, specifically the anterior pituitary and adrenal gland, is almost nonexistent. During this period of hyporesponsiveness, maternal separation for an hour or less does not elevate CORT or ACTH (Kuhn, Pauk, & Schanberg, 1990; Stanton, Gutierrez, & Levine, 1988). However, if the pup experiences longer periods of separation from the mother, it will exhibit elevated levels of CORT and ACTH, which will eventually return to basal levels upon reunion with the dam (Levine, 2001). Around PND 18, rat pups demonstrate an increase in plasma CORT and ACTH when separated from their mother (Hennessy, 1996). Thus, there is a period in which glucocorticoid levels are very low, and the rat pup is almost immune to the effects of brief stressors. This period of hyporesponsiveness (PND 4-14) may shield the developing brain from exposure to excess glucocorticoids (Walker, Perrin, Vale, & Rivier, 1986).

According to Levine (2001) and Hofer (1987) there are specific forms of maternal stimuli that regulate aspects of the pup's HPA axis, and the removal of these stimuli can result in the dysregulation of the axis. Maternal behaviors that influence the development of the HPA axis are licking, grooming, and arched-back nursing (Levine, 2001; Suchecki, Mozaffarian, Gross, Rosenfeld, & Levine, 1993). It has been reported that dams who spent more time licking, grooming, and arch-back nursing their offspring, had pups that grew up to be less fearful of novelty, compared to the offspring of mothers who did not groom and lick their pups as much (Caldji et al., 1998; Levine, 1994). As adults, the pups of these attentive dams also had a smaller CORT and ACTH response to stressors, and returned to resting levels of CORT and ACTH sooner than pups of less attentive mothers (Liu et al., 1997).

Though many studies have examined the effects of early stress in rat pups, it is difficult to draw conclusions across many of the studies. Researchers tend to vary in how they manipulate their experimental and control conditions. When examining the effects of separation, the mother or infant may be removed from the home cage, and the pup may or may not be left in contact with the other pups. Comparison groups also differ, yielding very different outcomes. Several studies utilize completely non-handled animals as a comparison group, while others use animal-facility-reared animals (exposed to handling during cage cleaning, feeding, etc). This therefore makes interpretation of the data very difficult. The studies that are summarized below provide a general overview of the results, while highlighting the conflicting data.

When pups are separated from their mother for brief periods of time during the first two weeks of life, in a paradigm termed “handling”, the HPA axis of the rat pup can be dramatically altered. Many studies have reported that animals handled for fifteen minutes per day mounted a smaller CORT and ACTH response when exposed to stressors as adults, compared to non-handled pups (Levine, Haltmeyer, Karas, & Denenberg, 1967; Meaney et al, 1993a; Meaney, Aitken, Viau, Sharma, & Sarrieau, 1989; Viau, Sharma, Plotsky, & Meaney, 1993). Handled animals not only exhibited a decreased magnitude in their stress response, they also returned to resting levels of CORT and ACTH faster than non-handled pups (Levine et al., 1967; Meaney et al., 1993b; Viau et al., 1993). Handled pups also exhibited reduced fearfulness and greater activity when placed in novel situations as adults (Levine et al., 1967). Meaney et al. (1989) found that handled rats had a much larger suppression of CORT secretion after administration of dexamethasone compared to unhandled rats. There were no differences between handled and unhandled rats when given CRF or ACTH. These results indicate that the negative feedback of glucocorticoids in handled rats was more effective than in the non-handled rats, but that the pituitary and adrenal gland were operating similarly. These differences in HPA activity persisted over the lifetime of the pup.

It is important to note that these results are not as robust when the comparison group consists of animal-facility-reared rodents (Heim, Owens, Plotsky, & Nemeroff, 1997; Walker, Welberg, & Plotsky, 2002). This indicates that the differences found between handled and non-handled rats may be partially due to the non-handled rats mounting a large HPA response in the face of a stressor, creating a large difference between the two groups. In other words, just as handled animals demonstrated lower stress responses, non-handled animals demonstrated larger stress responses due to a lack of stimulation in the non-handling paradigm. Handling appears to have “inoculated” the rat pup to later stress, while non-handling may have made the rat pup more vulnerable to future stress.

The time course of the handling does not appear to alter these outcomes. Pups handled for 15 minutes on PND 1-5 exhibited similar CORT and ACTH responses as pups handled on PND 1-20 (Hess, Denenberg, Zarrow, & Pfeifer, 1969). Likewise, pups that were handled on PND 1-20 exhibited a reduced HPA response in the face of stress just as those handled on PND

21-41 (Ader & Grota, 1969). Handled and non-handled rats did not differ on basal levels or the diurnal pattern of CORT or ACTH (Kaufman & Charney, 2001; Levine et al., 1967; Meaney et al., 1989).

There appear to be several factors that influence the outcomes described above. The behavior of the dam upon reunion with the rat pup may be responsible for the HPA alterations in the pups (Francis, Caldji, Champagne, Plotsky, & Meaney, 1999; Kaufman, Plotsky, Nemeroff, & Charney, 2000; Levine, 2001; Levine et al., 1967). Studies indicate that handling increased ultrasonic vocalizations in pups, which increased maternal care given to the pup upon reunion (Bell, Nitschke, Gorry, & Zachman, 1971). Dams of handled pups exhibited higher rates of grooming, licking, and nursing than dams of non-handled pups (Francis et al., 1999; Levine, 2000; Liu et al., 1997). Thus, after separation, dams exhibited higher rates of attentive behavior, which led to pups that were less responsive in the face of stress. On the other hand, non-handled animals received no external stimulation, and the mothers never left the nest. This lack of external stimulation and lack of brief periods of maternal absence may have sheltered the pup from mildly stressful experiences, making it vulnerable to stress later in life.

Longer periods of separation between the developing pup and dam lead to different developmental outcomes. Typically, dams and pups have been separated during the first 21 days, for 3-24 hours. These longer periods of separation caused pups to exit the period of hyporesponsiveness, and their CORT and ACTH increased during the length of the separation (Rosenfeld et al., 1991). Maternally deprived pups demonstrated a greater CORT response when given ACTH than non-maternally deprived pups (Rosenfeld et al., 1991). When given dexamethasone, maternally deprived rats suppressed their production of cortisol for shorter periods of time than non-maternally deprived rats, indicating a reduced negative feedback system

(Meaney et al., 1993b). These results suggest that the adrenal gland may be more sensitive to stimulation in rats that have experienced maternal deprivation, and that the negative feedback loop is inhibited. As adults, maternally deprived pups demonstrated greater basal and stress response levels of ACTH and CORT, compared to non-deprived pups (Kaufman et al., 2000; Levine, 2001; Schmidt, Okimoto, Dent, Gordons, & Levine, 2002). As adults, these animals also displayed higher levels of anxiety-like behavior, hypervigilance, mild cognitive impairment, and a proclivity for alcohol consumption (Ladd et al., 2000).

The maternal deprivation effects are dependent on the age at which the pup experienced deprivation. If maternally deprived on PND 3, the pup was hyperresponsive to stress when tested on PND 20 (Rots et al., 1996), but when deprived on PND 11, the pup was hyporesponsive to stress on PND 20 (Van Oers, de Klot, & Levine, 1998). Thus, the dams may have been responding differently based on the pups' age, behavioral stage of development, and/or physiological development (Sanchez, Ladd, & Plotsky., 2001). Some dams have displayed a delay in retrieving their pups, as well as a delay in licking, grooming, and nursing them after a lengthy separation, while others did not (Huot, Smith, & Plotsky, 1997; Plotsky & Meaney, 1993). Huot et al. (1997) posits that it is the quality of this reunion that determines the organization of the pups' HPA axis.

Finally, rats exposed to prolonged social deprivation after an early weaning (Day 15-16) exhibited more aggressive and anxious behavior than controls, but had a hypofunctional HPA axis (Sanchez, Aguado, Sanchez-Toscano, & Saphier, 1998). These animals had blunted basal levels of CORT, and a blunted response to stress. Thus, the developmental state of the HPA axis at the time of insult may also be a factor that determines the outcomes of the axis, either hyper- or hyporesponsiveness (Sanchez et al., 2001). These outcomes suggest that the primary caregiver and social environment are important factors in the development of the HPA axis. In rodents, how the dam cares for her pup upon reunion, and the levels of stress the pup is exposed to, appear to drastically alter the development of the pup's HPA axis.

APPENDIX C

LITERATURE REVIEW OF PRIMATE STUDIES

There have been over fifty studies that have examined the effects of early stress on the developing monkey. Most of these studies have examined the effects of stress on macaques, though there are a few which have also studied new-world monkeys. These studies can primarily be divided into two categories: 1) those that examine the effects of different rearing histories (i.e. surrogate-reared, isolation-reared, peer-reared), and 2) those that examine the effects of separation between the infant and the mother figure (biological or surrogate mother).

In addition to these two early manipulations, researchers have examined the short and long-term effects from many different angles. Monkeys have been examined at different ages, with differing lengths and frequencies of separation. The types of separations have also been manipulated in different ways, with both being separated from their social group, or with just one being removed. Rearing histories have been just as varied, with infants having been reared in complete isolation, isolation with periodic peer interaction, inanimate surrogate-reared, peer-reared, dog-reared, or mother-reared in different foraging demands (different levels of stress on the mother). Measures of HPA activity differ among studies too, with either saliva, plasma or cerebrospinal fluid being obtained. There are as many different outcomes as there are manipulations to these experiments. I will review a selection of these different studies, discussing the most consistent behavioral and physiological outcomes.

Harry Harlow and his colleagues examined the effects of early mother-infant separation by raising rhesus macaque (*Macaca mulatta*) infants in isolation, with peers, or with their mothers. In general, infants raised in isolation demonstrated higher rates of aggression, repetitive movements (pacing, rocking, circling), vacant staring, unresponsiveness, self-clutching, and increased non-nutritional sucking compared to normally raised infants (Harlow & Harlow, 1969). Harlow, Harlow, and Suomi (1971) reported that infants raised in isolation failed to play, contact other animals, or engage in appropriate sexual behavior. These results were still evident up to three years later.

Isolation-reared female monkeys frequently rejected their own infants, cradled them clumsily, and tolerated little ventral contact or nursing (Seay, Alexander, & Harlow, 1964). Half of these mothers were also abusive: crushing, dragging, throwing, and jumping on their infants. The abused infants of these motherless mothers approached their mothers more frequently and showed more nonspecific contact with them than did the control infants (Seay et al., 1964). The motherless mother infants demonstrated delayed peer interactions for the first four months of life (Harlow, Harlow, Dodsworth, & Arling, 1966).

Sackett, Bowman, Meyer, Tripp, and Grady (1973) continued to examine the effects of isolation rearing, by analyzing the behavioral and neuroendocrine responses of rhesus infants that were either peer-reared or raised in isolation for the first nine months of life. The isolation reared monkeys demonstrated more fearful behavior, and had higher basal plasma cortisol than the peer-reared animals. Thus, it was demonstrated that isolation could have profound effects on behavior and HPA activity, which continued into adulthood (but see Meyer & Bowman, 1972, who found no effects of isolation on cortisol levels in 4-year-old animals).

Researchers have also evaluated the effects of peer rearing on the developing monkey. Champoux, Metz, and Suomi (1991) examined peer-reared and mother-reared rhesus infants from 0-5 months of age, and again from 10-21 months of age when all were integrated into social groups. During the first five months, the mother-reared infants spent more time nursing, sleeping, and clinging to their mothers, while the peer-reared infants spent more time having spasms, vocalizing, locomoting, and exploring. Once the two groups were integrated into social groups at six months of age, they did not differ behaviorally. However, Clarke (1993) conducted a similar study, and found that peer-reared animals were more active after the group change (no other behavioral measures were significantly different), and that they had lower plasma cortisol and ACTH measures. Ruppenthal, Arling, Harlow, Sackett, and Suomi (1976) found that peer-reared adults showed less sexual behavior and play than mother-reared, and that they were more aggressive and hostile. These studies indicate that the behavior of peer-reared animals is altered early in life and that the responses of their HPA axis may be blunted.

In a similar vein, Boyce, Champoux, Suomi, and Gunnar (1995) examined the salivary cortisol of nursery-reared rhesus infants throughout the day, to determine if they demonstrated an altered circadian rhythm compared to mother-reared animals. The animals were between 4 and 5 months of age, and were exposed to interactions with peers on some of the testing days. They found that the nursery-reared animals had a peak in their cortisol at 11 a.m., which was shifted from the expected peak at 8 a.m. (one hour after lights-on) exhibited in mother-reared animals. The authors suggested that the infants may have been phase-delayed in their HPA rhythm, which, interestingly, is also the case in some patients with anxiety disorders.

Other studies have examined the effects of separation from a “mother-figure” on infants with different attachment objects (mothers, surrogates, or peers). Yvette Spencer-Booth and Robert Hinde (1971a, b) evaluated the effects of mother-infant separation in socially housed rhesus infants. Mothers were removed from their infants when the infants were between the ages of 4.5 and 8 months, for a period of 6 to 10 days. During the separation, infants exhibited behavior indicative of stress: increased vocalizations and motor activity, followed by decreased motor activity, and a slouched posture (referred to as the agitation/depression reaction) (Spencer-Booth & Hinde, 1971a, b). During the period of separation, the infants rarely played, and they ate and "whoo" called more. Once the infants were reunited with their mothers, they spent more time on her than before the separation. At twenty-four months of life, these infants were still less active than controls, and engaged in less social play and more manipulative play. These studies were the first to detail the behavioral disturbances of separation on the developing rhesus, and demonstrated that the effects of this stressful experience were noticeable two years later.

Similar outcomes have also been obtained in other species of socially housed monkeys. Kaufman and Rosenblum (1967) examined the effects of a four-week removal of pigtail macaque (*Macaca nemestrina*) mothers on their infants. The infants displayed the typically agitation/depression response during the first week of separation, with less play and interaction with others during the depression phase. Activity increased after a week, though the infants still showed minimal signs of depression. The infants did not interact much with others during the separation. The authors did not report any long-term effects.

Levine and his colleagues (1984, 1987, 1988) examined the behavioral and physiological outcomes of separation with the mother absent and with the mother nearby. When 4-6 month old rhesus infants were separated from their mother, who remained in visual or limited tactile contact, they behaved differently than when separated from her completely. These infants were more active, "whoo" called more, and did not demonstrate depression. The infants that were in complete isolation often demonstrated the agitated/depressed response. Levine and Wiener (1988) suggested that when the mothers were partially present, the infants emitted signals that were meant to cause her to retrieve them. Because the mothers were always there, the infants did not enter the depressed mode, but instead remained agitated because their mothers were not responding to their cues. When the infants were in partial contact with their mothers, they had very little HPA activation. Though the behavior may have indicated "stress", they were not physiologically aroused. Levine and his colleagues (1984, 1987, 1988) argued that the mothers were buffering the stress response of the infants (maternal buffering). The infants that were in complete isolation had a lower behavioral response, but their plasma cortisol levels were high. Thus, it appears that when the infant was in partial contact with the mother during separation, it was more behaviorally active than the infant that was in complete separation, however its HPA axis did not show signs of stress. The infant appeared to be using vocalizations to draw the mother close to it, but its HPA axis was not activated because the mother was still nearby (Levine, 1987; Levine, Franklin, & Gonzalez, 1984; Levine, & Wiener, 1988).

Meyer, Novak, Bowman and Harlow (1975) examined the effects of separation on rhesus infants that were either mother-reared or surrogate-reared. At six months of age, infants were separated from their "mother" for nine weeks. The mother-reared infants were more affected by the separation than the surrogate-reared infants, though both demonstrated changes in behavior. Both had higher rates of vocalizations and locomotion, and lower levels of play on the first day after the separation. The mother-reared infants played less and demonstrated more stereotypies. Kraemer, Ebert, Schmidt, and McKinney (1991) also found similar results when they compared mother-reared, stationary surrogate-reared, and rocking surrogate-reared monkeys. The mother-reared infants again had the most severe reaction to separation, with higher rates of locomotion, vocalizations, and stereotypies at separation. The authors conclude that the mother-reared animals were more severely affected by the loss of their attachment figure than the surrogate-reared animals.

Higley, Suomi, and Linnoila (1991) compared the effects of several separations across the first two years of life on rhesus infants that were peer-reared during the first six months to infants that were mother-reared the first six months of life and then placed with peers. They found that peer-reared animals had higher basal plasma cortisol across the first two years (also see Champoux, Coe, Schanberg, Kuhn, & Suomi, 1989 for similar basal cortisol results). During the separation tests, the peer-reared infants had higher plasma cortisol. Similarly, Fahlke et al. (2000) found that six-month old peer-reared rhesus infants had higher cortisol in response to separation than mother-reared infants. The authors suggested that the peer-reared infants may have had higher levels of fearfulness, and that they may have been in a state of chronic anxiety.

In contrast, Clarke, Kraemer, and Kupfer (1998) examined the effects of a one-week separation on mother-reared and peer-reared rhesus monkeys when they were 10-months old, and found that the mother-reared infants had higher cortisol and ACTH before and after the separation. The peer-reared infants appeared to be hyporeactive to the stress of separation. Likewise, Shannon, Champoux, and Suomi (1998) found, that in response to separation, mother-reared infants had higher cortisol compared to peer-reared and surrogate-reared infants.

Finally, in an interesting design, Rosenblum (1987) and Coplan et al. (1996) examined the effects of different stressors on bonnet macaque (*Macaca radiata*) and rhesus macaque mothers as a way of assessing the effects on the developing infants. Mother-infant pairs either experienced a low foraging demand (LFD)--food was readily available, a high foraging demand (HFD), or a variable foraging demand (VFD). The infants in the VFD had more unstable relationships with their mothers (higher number of contacts made and broken), they played less with others, and they had more vocalizations, fear grins, and cringes. Coplan et al. (1996) found that as adults, the VFD monkeys had higher levels of CRF and lower levels of cortisol, as measured by cerebrospinal fluid, than the other groups. Rosenblum (1987) postulated that the reason the infants in the VFD condition were most affected was because the mother was there physically, but not psychologically. The VFD mothers were in a constant state of stress, focusing their energies on obtaining food, and therefore did not have quality interactions with their infants. This resulted in infants who demonstrated anxiety-like behavior, who did not know how to relate with others, and who possibly had an altered HPA axis.

Only a handful of studies have examined the effects of various pharmacological tests on primates living in stressful environments. Brooke and colleagues (1994) administered dexamethasone to cynomolgus macaques (*Macaca fascicularis*) living in stable and unstable social groups. They discovered that the animals that were resistant to dexamethasone suppression came from unstable groups. Johnson et al. (1996) examined the effects of dexamethasone and CRF in a group of marmosets (*Callithrix jacchus jacchus*) that experienced all of the following living conditions: 1) stable social group, 2) unstable social group, 3) heterosexual pairs, 4) isolation. While the marmosets were in isolation and when they were in an unstable social group, they were much more resistant to dexamethasone suppression, secreting higher levels of plasma cortisol in comparison to the other groups. While the marmosets were in isolation they exhibited lower levels of plasma ACTH and cortisol when given CRF, whereas under the other conditions they exhibited large levels of ACTH and cortisol. These results indicate that while under stressful conditions, the HPA axis of animals is much less sensitive to synthetic glucocorticoids, such that negative feedback is reduced. The results also suggest that when stressed, the pituitary gland, and potentially the adrenal gland, are less sensitive to the CRF test.

There are only a few studies that have examined the behavioral outcomes of maternal maltreatment in non-human primates. Maestripieri and Carroll (1998) examined the effects of maternal maltreatment in pigtail macaques. Abusive pigtail mothers had controlling parenting styles, meaning that they rejected their infants and protected them more than control mothers (Fairbanks, 1996). Compared to controls, the abused pigtail infants demonstrated play at a later age, played less, and initiated play bouts less often. Maestripieri, Jovanovic, and Gouzoules (2000) also found that abused rhesus infants were slower at gaining independence from their mothers, and spent more time on their mother during the first two months of life. McCormack (2000) found that a group of abused rhesus infants screamed more, and played less and at a later developmental date than non-abused infants.

It is clear from the research reviewed that early stressors imposed on the developing primate infant can have profound effects on the behavior and neuroendocrine system. The early research on isolation has demonstrated that a lack of maternal contact and/or a normal mother-infant relationship prevents infants from learning appropriate social skills to apply to their interactions with others. These infants did not interact with others appropriately; they demonstrated fearful behavior, and had higher levels of cortisol. Infants that experienced separation from their attachment figures often demonstrated an agitated-depressed reaction and an increase in cortisol during the separation, followed by an increase in contact with the mother, and a decrease in interaction with others upon reunion. The HPA axis responded differently to separation, depending on the infant's rearing history. Several studies indicated that the HPA axis of peer-reared infants was hyporeactive, while others indicated that it was hyperreactive. Neuroendocrine challenges suggest that stress may inhibit the negative feedback system, and may impact how the pituitary and adrenal gland respond. Altogether, the data suggest that adverse conditions between an infant and its mother provoke behavioral and physiological responses in the infant that depends on the age of the infant, the context of the adversity, and the duration of the adverse condition.

APPENDIX D

LITERATURE REVIEW OF ATTACHMENT THEORY

John Bowlby’s theory of attachment predicts many of the behavioral outcomes in individuals who have had altered relationships with their caregivers, and Gary Kraemer’s psychobiological theory of attachment offers a compelling explanation for the physiological outcomes observed in these children. Attachment theory holds that the child’s formation of an affective bond with a primary caregiver (usually the mother) is critical in the first year of life (Bowlby, 1958, 1969, 1973, 1980). The function of attachment is to increase the infant’s chances of survival (Bowlby, 1969). The infant exhibits behavior patterns that keep it in close contact with its caretakers. Examples of these attachment patterns (also referred to as proximity signals) are clinging, grasping, returning to the caretaker, crying, smiling, and babbling (Frodi, Lamb, Leavitt, & Donovan, 1978). According to the theory, adults too are biologically predisposed to maintain proximity and protect the infant. Thus, both adults and infants act in ways that increase the number of interactions the infant has with particular adults, ensuring the infant’s survival.

As the infant matures, it begins to explore and learn about the environment. It is during the second half of the first year of life that the attachment relationship truly develops between the infant and primary caregiver(s). This coincides with the development of locomotion, allowing the infant to explore the environment while maintaining proximity to caregivers. Attachment theory predicts that during times of danger, the infant will seek out and maintain protective proximity to the attachment figure. Proximity signals may also be activated when the caretaker is too far away or has been away from the infant for an extended period of time (Crittenden & Ainsworth, 1989).

According to Bowlby (1969), the quality of the attachment bond depends on the appropriateness and sensitivity of the adult's response to the proximity signals of the infant. Infants become securely attached to caretakers who consistently and appropriately respond (by meeting the needs of the infant) to their attachment signals. A secure attachment between a mother and infant is said to occur when a mother is sensitive to the needs and signals of the infant (Ainsworth, 1973; Ainsworth, Blehar, Waters, & Wall, 1978). A secure infant is confident that its caretaker will meet its needs, and that it is safe to examine the environment. The infant's sense of security influences whether or not it is safe to leave the caregiver to explore the world (Ainsworth, 1973). A child who is not securely attached, does not feel confident in the caretaker's ability to protect it, and is less likely to leave its caretaker and explore compared to the securely attached child.

In addition to allowing infants to explore, a secure attachment is also thought to promote competence in peer relations via the child's internal working models of self, other, and relationships (Bowlby, 1969, 1973, 1980). A child that has experienced a secure attachment is thought to internalize a sense of others as being available, and of the self as being worthy of care (Mueller & Silverman, 1989). In this way, the child can enter his social world with the expectations that others will be responsive and that interactions will be pleasurable. Children that have had an insecure attachment bond may not be able to interact with and trust others in the same way. They may be hesitant to interact with their peers, and have great difficulty in establishing peer relationships. Thus, the quality of the infant-caregiver relationship affects the quality of future relationships between the child and others.

Empirical evidence suggests that there are differences in the quality of interactions between mothers and their infants (Ainsworth et al., 1978). When securely attached infants are stressed, they quickly seek their attachment figure. These infants are easily calmed and reassured by their caretaker, and are ready to explore their environment again. Insecurely attached infants are highly distressed when separated from their mothers, and when the mothers return, the infants behave in an ambivalent manner and fail to be comforted by her.

Attachment theory predicts that maltreated infants will be more likely to demonstrate insecure attachments with their primary caretakers, and this is evident in the literature (Cicchetti, 1989). Maltreated infants are more likely to be classified as insecure in their attachments to their primary caregiver (70-100%), than are children who have not been maltreated (39%) (Cicchetti & Braunwald, 1984; Crittenden, 1985, 1988; Egeland & Sroufe, 1981; Gordon & Jameson, 1979; Lamb, Gaensbauer, Malkin, & Schulz, 1985; Lyons-Ruth, Connell, Zoll, & Stahl, 1987).

Attachment theory predicts many of the behavioral outcomes in maltreated children, but it does not predict physiological outcomes. Gary Kraemer recognized this, and modified Bowlby's theory to include explanations of physiological alterations that may occur in response to abnormal mother-infant relationships (Kraemer, 1992; 1997; Kraemer et al., 1991). This theory is called the psychobiological attachment theory (PAT).

Basic attachment theory assumes that the regulation of the infant's physiology is a product of genetic endowment. PAT argues that it is also due to maternal regulation (Kraemer, 1997). In support of this, Hofer (1987) and Levine (1994) have demonstrated that the biological mother has a significant effect on the neurobiological characteristics of the infant. Suomi (1999) also argues that rhesus monkey mothers entrain their infants to their biological rhythms via extended ventral-ventral contact, and their offspring typically develop synchronous rhythms. As a result, Kraemer (1997) suggests that the mother, and the infant's attachment to her, are responsible for the development of the infant's neurobiological systems, and regulation. PAT holds that the mother provides an external regulatory mechanism for the infant's systems, because the infant cannot regulate all systems on its own right after birth. This process is called attunement (Field, 1985). With time, the infant begins to self-regulate (Kraemer et al., 1991).

Unlike Bowlby's attachment theory, which posits that individuals cannot behave competently in their environment and cope with stressors without adequate working models, PAT suggests that the neurobiological systems of the infant develop in sequence and in relation to the caregiver, and that individuals cannot behave competently until these systems are in place (Kraemer, 1992). The attunement between the mother and the infant will impact the degree to which the infant will come to regulate its behavior and physiological arousal later. The regulatory capabilities of the infant can be compromised if the caregiver fails to provide an adequate physiological model for the infant (Kraemer, 1992).

The development of the HPA axis can be dramatically altered because of the mother-infant relationship (Boyce et al., 1995; Gunnar et al., 2001; Rots et al., 1996; Van Oers, de Klot, & Levine, 1998). PAT predicts that secure infants would be able to appropriately regulate in the face of stress, and would exhibit a different HPA response than infants with insecure attachments. PAT predicts that secure infants would have an initial stress response when faced with a stressor (an increase in cortisol), but that with time, the infant would be able to moderate it's response (lowered cortisol over time). Insecure infants would not necessarily have the same response, depending on what the infant had learned from the mother. The insecure infant could do one of two things in the face of stress: 1) demonstrate an increase in cortisol at the onset of the stressor, with a high level of cortisol secreted throughout the stressor because the infant has not learned how to cope and self-regulate, 2) have no increase in cortisol because it has not learned the appropriate response to stress, or because the system has begun to down-regulate in response to constant stressors earlier in life. Both of these responses have been observed in children who have experienced early adverse experiences (Gunnar & Vazquez, 2001; Marsland et al., 2000). Whether an insecure child will be hyperresponsive or hyporesponsive in the face of stress will be based on several factors: 1) the timing of the stressor, specifically the stage of development that the HPA axis is in when stressors began, 2) the type and magnitude of the stressors, and 3) the resources and coping skills already available to the individual when stressors began (Kraemer, 1997; Sanchez et al., 2001). In any case, if there is a disruption between normal mother-infant interactions, the HPA will become dysregulated (Sanchez et al., 2001).

APPENDIX E

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