Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Matthew McMurray 4/18/2012

The Effect of Adverse Maternal Care in Socially Housed Rhesus Macaques

by

Matthew McMurray

Sarah Gouzoules
Adviser

Department of Anthropology

Sarah Gouzoules

Adviser

Mar Sanchez

Committee Member

John Kingston

Committee Member

2012

The Effect of Adverse Maternal Care in Socially Housed Rhesus Macaques

By

Matthew McMurray

Sarah Gouzoules

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Arts with Honors

Department of Anthropology

2012

Abstract

The Effect of Adverse Maternal Care in Socially Housed Rhesus Macaques
By Matthew McMurray

In this study, rhesus macaques (*Macaca mulatta*) were used to study differences in rates of infant exploration, vocalization, and attachment to the mother between maltreated and control infants. Results indicated that maltreated infants are more behaviorally inhibited and less likely to call for help compared to control infants. Males also engaged in more exploration away from the mother compared to females who, in turn, had higher rates of proximal exploration. These findings are valuable because they suggest that there are early behavioral differences between maltreated and control infants.

The Effect of Adverse Maternal Care in Socially Housed Rhesus Macaques

By

Matthew McMurray

Sarah Gouzoules

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Arts with Honors

Department of Anthropology

2012

Acknowledgements

I would like to thank Dr. Mar Sanchez and members of her lab, especially Brittany Howell, at the Yerkes National Primate Research Center Field Station, Lawrenceville, Georgia for their help and guidance in collecting and analyzing data. I would also like to thank Dr. Sarah Gouzoules for her patience and assistance throughout the Honors program.

Table of Contents

Background

Rhesus macaques (*Macaca mulatta)* provide a good animal model to study the effects of early environment on development in humans due to the social, emotional, physiological, and neurological similarities between species. In addition, rhesus monkeys are most commonly used to study the impact of early social experience because they adapt well to different environments, including captivity and laboratory conditions (Sanchez 2006). Both rhesus monkeys and humans also share similar patterns of infant attachment to caregiver (Bowlby 1969) and development (Suomi 2005; Moriceau & Sullivan 2005).

The social structure of rhesus monkeys also makes them advantageous for behavioral studies as a result of their strong dominance hierarchies organized around matrilines where the offspring inherits the mother's social rank (Suomi 2005). The females remain in their natal troop and matriline their entire lives, maintaining a stable social status, with the exception of rare overthrows, while males migrate as individuals organized into bachelor groups and join new troops after reaching puberty (Campbell et al. 2011). Relative social status within a troop is mostly dependent on mutual social support from allies (friends and relatives) rather than on individual size or strength (Campbell et al. 2011).

Mother-infant pairs stay in close physical contact for a year or until the mother begins to wean the infant in anticipation of having another infant (Campbell et al. 2011). After two months, infants begin to explore their environment and increase play with peers and other social interactions (Hinde & Spencer-Booth 1967). The juvenile period, generally defined as one year to three or four years of age, is a time of increased interaction with peers while also maintaining a close relationship with the mother. During the juvenile

period, the mother still acts as a safe base in times of stress and as a protector in social interactions with other animals (Suomi 2005).

Rhesus macaques and humans share similarities in stress-related behavioral and endocrine responses as well as in brain structure (Kalin & Shelton 2003). Monkeys, like humans, exhibit similar distress responses during maternal separation, such as vocalizations (Sanchez et al. 2005). In the brain, the hypothalamic-pituitary-adrenal (HPA) axis is the main system that mediates neuroendocrine responses to stress that result in the release of glucococorticoids, cortisol in primates (Sanchez 2006). This process begins after perceiving a stimulus as threatening. Then the information is encoded in different areas of the central nervous system and funneled to the hypothalamic paraventricular nucleus which stimulates the secretion of corticotrophin-releasing factor (CRF) and vasopressin (AVP). These neuropeptides are then transported to the anterior pituitary which stimulates the secretion of adrenocorticotrophic hormone (ACTH) into the systemic circulation which, in turn, stimulates the secretion of glucocorticoids from the adrenal cortex until the circulating glucocorticoids complete the negative feedback loop to shut off the current HPA axis activity (Sanchez et al. 2001). The function of the HPA axis is important to understand in that it controls the release of cortisol, the stress hormone. Having either higher or lower amounts of cortisol than average has been associated with psychiatric and somatic illnesses including depression, and posttraumatic stress disorder (PTSD), among others (Sanchez 2006).

Introduction

Maltreatment in Humans

The differences between maltreated infants and control infants reacting to novel environments can help researchers understand how child abuse and neglect may negatively affect infant development. In 2003, more than 3 million children were reported to be victims of parental maltreatment (Hussey et al. 2005). In addition to the obvious risks of injury and death, early childhood maltreatment has been shown to increase the risk of developing physical and psychiatric disorders in adults and future generations (Champagne & Curley 2009). In addition, there is the social issue of perpetuation of maltreatment from generation to generation. Thus, while females who were abused in childhood do not always become abusive mothers, the risk of abuse to their own children is much higher, particularly if the mother is young (de Paul & Domenech 2000).

There have been many studies examining abuse, particularly sexual abuse, but the understanding of how neglect impacts development is less well known. Neglected children often are described as more anxious/avoidant, exhibit delayed social development, have difficulty differentiating facial expressions, and are typically more passive when interacting with their peers than normal children (Crittenden & Ainsworth 1989; Sanchez & Pollak 2009). Crittenden (1986) found that neglected children as adults find it difficult to leave home and form new friendships and attachments. In fact, Allen & Oliver (1982) discovered that neglect alone hindered language development more than abuse. Neglect can also severely affect children in school, particularly middle school, and alter their academic achievement and behavior in the class (Kendall-Tackett & Eckenrode 1996). Leiter and Johnson (1994) hypothesized that these learning deficits were a product of neglectful

parents who would be less likely to read to their children and support the child with their work. Individuals who were neglected as children have been found to want the comfort and support of others but do not seek it out or are not comforted once they receive it (Crittenden & Ainsworth 1989). In addition, adults who were formally maltreated as infants often find it difficult to form a secure relationship with their own child, which may explain why maltreatment continues to be passed on from one generation to the next (Ricks 1985). This neglectful behavior therefore seems maladaptive for children because it not only leads to increased risk for emotional alterations (increased reactivity, anxiety, fear, and depression), but will also affect their social behavior and formation of attachments with peers, partners, and their future offspring (Morton & Browne 1998). Egeland (1991) found that this vicious cycle can be stopped in humans if they had some regular exposure to a positive caregiver and they were willing to acknowledge that they were maltreated and challenge their former representational model of attachment (most of the times reactive, avoidant or disorganized).

Attachment in primates is vital to survival during infancy (Crittenden & Ainsworth 1989). John Bowlby (1969) was one of the first attachment theorists and described mother-infant attachment as a lasting connectedness that has a large impact throughout life. If the mother is present and is available and responsive to the infant's needs, a child will leave the mother and explore the environment (Bowlby 1977). Vocalizations such as coo calls and screams are common infant solicitations for retrieval/help and function to express alarm or stress; infants typically cease calling once the mother moves into proximity or contact with the infant (Sugiura 1997). Mothers act as a safe base that gives children a sense of security and a safe place from which to explore the environment. Infant

attachment is not present at birth but natural selection appears to have favored the development of behaviors to make the infant automatically try to form a bond or maintain proximity with their caregiver (Crittenden & Ainsworth 1989; Morton & Browne 1998). The attachment relationship is important around the time of first locomotion in infants, when they begin interacting and exploring new environments by themselves (Morton & Browne 1998). Insensitive behaviors from the mother toward a child do not negate an attachment relationship, but rather lead to infants feeling insecure, reactive or avoidant around the primary caregiver. The child will then form a representation of the primary caregiver as being unresponsive and unavailable, with the child feeling not worthy of the caregiver's attention (Morton & Browne 1998).

One of the most important factors supporting normative primate (including human) development (social, emotional, and relationship with the environment) is attachment to the caregiver. In an orphanage it can be difficult for an infant to form a bond with a caregiver with multiple caregivers rotating. Without the proper attachment, an infant can suffer detrimental emotional effects (elevated fear and anxiety), social deficits (poor attachment to other people), detrimental cognitive effects, and can be more prone to addiction (Sanchez et al. 2010; Williamson et al. 2003; McCormack et al. 2006). Orphanage rearing in poor conditions, specifically in Romania, has provided examples of poor social stimulation and reduced opportunities for infants to form attachments with a caregiver (Gunnar & Donzella 2002). Orphanage reared-children did not show normal daily cortisol levels and many had lower than expected morning levels compared to the average (Gunnar & Donzella 2002). Interestingly, once these children were adopted and were able to form proper attachments with a caregiver, they had closer to normal levels of cortisol. The

length of time in the orphanage (not proper attachment) provided a reliable predictor of how long the child would have elevated cortisol after leaving (Gunnar & Donzella 2002).

Behavioral inhibition in children can have effects on stress regulation and social adaption. Nacmias et al. (1996) defines behavioral inhibition as, "the tendency to restrain or restrict one's approach to new people, events, and/or objects." Behaviorally inhibited children are more likely to continue having an inhibition as adults and are at increased risk for internalizing disorders, such as anxiety (Degnan & Fox 2007). Inhibited children perceive novel events as threatening and have exaggerated reactions to stress. It is thought that inhibited children have enhanced amygdala activation in response to novelty or strange events (Nachmias et al. 1996). The availability and behavior of caregivers play a role in children's reactions to novel situations and the security of the attachment influences the child's feeling of protection (Nachmias et al. 1996). Highly protective parents who overly react to their child's fears are actually reinforcing the fears and tend to maintain behavioral inhibition through childhood (Degnan & Fox 2007). However, some researchers have hypothesized that intervening factors can change a child's inhibition and achieve positive adaption despite experiencing past threats (Luthar et al. 2000; Degnan & Fox 2007). Some young children who are behaviorally inhibited are also able to interact socially and can lower the chances of developing anxiety disorders. If a child is able to adjust to novel situations it can help build resilience to help control future anxiety provoking situations.

When growing up, different types of stressful stimuli and situations are typically experienced. Intermittent stress, including mildly stressful early experiences, may actually promote the development of resilience and help juveniles to learn how to cope with future

stressors (Parker et al. 2004; Shonkoff 2005). In some cases these stress responses can have positive effects, not only because the infant learns strategies to adapt and handle the situation, but because it also leads to habituation of the neuroendocrine stress response by the HPA and the sympathetic systems.

Elevated cortisol levels have been found in some studies to have long term effects on numerous physiological processes. In human children, elevations in cortisol can affect internalizing problems, behavioral inhibition, social wariness, and withdrawal (Essex et al. 2002). If children experience extreme stress during infancy they are more vulnerable to be predisposed to alterations in HPA function later on, but if they are exposed to high levels of stress later in life they may not have abnormal cortisol levels (Essex et al. 2002). Both humans and rhesus monkeys have periods of high plasticity in the brain during development; however, humans appear to have a wider window of ontogenic vulnerability to negative behaviors (O'Connor & Cameron 2006). Genetics appear to influence the exact timing of brain development and may explain why some children are able to outgrow elevated cortisol levels and others retain increased HPA activity, for example.

Maltreatment in Non-human Primates

One of the benefits of using rhesus macaques for this study is that their social complexity, strong mother-infant bonds, and similar brain structure allow us to address questions that cannot be otherwise studied with human populations. Also, animal studies allow for high experimental control and the design of longitudinal studies that would be impossible to perform in humans. In particular, the use of prospective, longitudinal studies in populations of maltreated children is not feasible, due to the limitations of measuring and identifying rates, timing, and even severity of abuse and neglect in the home. The fact

that spontaneous infant maltreatment occurs in nonhuman primate species both in captivity and in the wild, with rates similar to those seen in humans at 2-5% (Maestripieri 2005), provides a unique experimental opportunity to address these questions in other primate species. In large populations of rhesus macaques living in captive groups, higher rates of abuse have been found, ranging from 5-10% (Maestripieri & Carroll 1998).

The subjects in this study are six-month old rhesus infants, some of whom were maltreated by their mothers. At the Yerkes Field Station of the Yerkes National Primate Center rhesus macaques, pigtail macaques (*Macaca nemestrina*), and sooty mangabeys (*Cercocebus atys*) have been observed to exhibit maternal abuse and rejection of their infants (Sanchez et al. 2010; Maestripieri & Carroll 1998; Maestripieri, Wilson, & Carroll 1997). Maternal abuse in rhesus monkeys is defined as, "violent behaviors [exhibited by the mother towards the infant] including infant dragging, crushing, throwing, stepping, or sitting on it" (McCormack et al. 2009). For the purposes of this study, subjects in the maltreatment group were included in that group (inclusion criteria) if they received at least three instances of violent infant-directed behavior within the first two weeks after birth: "1) dragging (*mother drags infant by its tail or leg while walking or running*), 2) crushing (*mother pushes infant against the ground with both hands*); 3) throwing (*mother throws infant at a distance while standing or walking*); 4) stepping or sitting on (*mother steps on infant with one foot or both feet*); 5) rough grooming (*mother pulls out infant's hair with force causing distress calls)*" (McCormack et al. 2006 p. 539). In addition to violent infant-directed behavior, a mother also had to exhibit high rates of early infant rejection (during the first three months of life), preventing the infant's attempts to get on nipple or in contact with the mother. Initially infant abuse was thought to be a form of aggressive

behavior. Then researchers found that in some cases the mother was treating their infant like an inanimate object without showing signs of aggressive arousal (Tsori & D'Amato 1983; Maestripieri et al. 2005).

There is evidence supporting that the transmission of abusive parenting is most likely the result of early experience. For individuals who were abused early in life, estimates of them becoming abusive parents are as high as 70% (Maestripieri 2005). Infant abuse is also often concentrated in matrilines and among closely related females (Maestriperi & Carroll 1998). In order to examine whether the trans-generational transmission of abusive maternal behavior was due to genetic or experiential factors, Maestriperi (2005) used a rhesus monkey cross-fostering design close to birth with more than half of the females who were reared by abusive mothers later abusing their own infants, while none of the females reared by control mothers did. These findings suggest that early experience, not heritable factors, is highly responsible for the perpetuation of abuse across generations.

In all primates, the ability to handle threatening and stressful situations is important for survival. In fact, some stress exposure early in life seems beneficial for later adaption, as demonstrated by reports that infant monkeys that experience some mild intermittent stress were found to more quickly initiate exploration compared to monkeys that were not previously subjected to stress (Parker et al. 2004). However, because in mammal species with strong mother-infant bonds the stress "inoculation" involves the regulation (buffering) of infant stress responses by the presence of the mother(Sanchez 2006), if there is no safe environment for the infant to go to or the stress is overwhelming, then the experience becomes detrimental (Schonkoff 2005).

Experiments testing Cerebral Spinal Fluid (CSF) have been used to examine whether abusive behavior is biologically and genetically determined or influenced. Maestripieri and colleagues (2006) found lower concentrations of serotonin (5HT) and dopamine metabolites from age one through three in abused infants compared to controls as well as in cross-fostered infants who were reared by an unrelated female. In this same study there were no significant differences in CSF concentrations for 5-HIAA (the main 5HT metabolite, used as a marker of 5HT turnover) with different alleles for the serotonin transporter gene (short-more vulnerable to dysregulation; long- more likely to have normal HPA axis). The fact that there was no difference between abused infants reared by their biological mothers versus cross-fostered infants suggests that differences in brain 5HT function are a result of early experience and not genetic similarities between the mother and offspring (Maestripieri et al. 2006). Interestingly, in other studies, no alterations in brain 5HT were found between abused and non-abused animals (Maestripieri & Lindell 2005; Sanchez et al. 2007). However, since abuse is highly correlated with higher rates of infant rejection very early in life, those studies also examined the effects of high maternal rejection (not just abuse) on the development of brain 5HT function. When measuring 5-HIAA in infants who experienced high levels of rejection, noncross-fostered and cross-fostered infants had lower CSF levels at age two than controls showing no direct signs of genetic influence (Maestripieri et al. 2006). Therefore, the maternal style, not the serotonin transport gene, was the major influence on how females reared by rejection mothers would later rear their infants. Low rates of CSF 5-HIAA are associated with higher anxiety, impulsivity, risk-taking behavior, and increased engagement in aggression in monkeys (Maestripieri et al. 2006).

Laboratory Temperament Assessment Battery Test

In humans, the Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith & Rothbart 1991) has been used not only to study individual differences in innate temperament, but how temperament and other aspects of behavioral reactivity to novelty and secure base behavior can be affected by early experiences, including maternal maltreatment. This battery of tasks has also been adapted for use with infant rhesus monkeys by Dr. Judy Cameron's laboratory at the Oregon National Primate Research Center, for 3-12 month old infant rhesus monkeys (Bethea et al. 2004; Cameron et al. 2003; Williamson et al. 2003).

The present study used the Laboratory Temperament Assessment Battery test to examine whether there were differences in temperament and behavioral inhibition between abused infants and control infants. All of the infants in this study were cross-fostered (control-abused, abused-control, control-control, abused-abused; see Methods below). In experiments examining temperament and behavioral inhibition, differences in exploration, fear, distress, and reactivity to novelty are commonly explored under low threatening conditions (Aksan & Kochanska 2004). Fox (2004 p. 171) defines temperament as, "the behavioral style of an individual that is present from birth, fairly stable across development, and influential in the formation of adult personality." Differences in temperament are easily detectable during the first few months of human life and are associated with patterns of social behavior during childhood. However, early life experiences, including early maltreatment, can modify innate temperament, reactions to novelty, and exploration. Children with high behavioral inhibition have been found to exhibit withdrawal during childhood and possibly develop psychopathology (Fox 2004).

Children with a negative emotional temperament have been shown to have larger increases in cortisol over the day, especially when subject to low quality childcare (Gunnar & Donzella 2002).

Differences in temperament or behavioral inhibition in rhesus monkeys have been found to be a result of differences in the function of limbic brain regions such as the amygdala and peripheral physiological functioning (Kalin et al. 1998). Human studies have demonstrated that individual differences in these limbic brain regions are associated with different emotional and temperamental types. Thus, in individuals with higher right/left prefrontal activity, this asymmetry is associated with increases in negative affect (Kalin et al. 1998). In studies combining the analysis of levels of cortisol with comparisons of brain asymmetry, Kalin et al. (1998) found that monkeys with greater left frontal lobe activation had lower levels of cortisol, while those with higher right activation had higher cortisol levels. These results possibly suggest that primates, both humans and non-humans, with greater left frontal lobe activation may also have lower rates of fearfulness, anxiety, and stress reactivity (both behavioral and hormonal).

Humans and rhesus exhibit similar prevalence rates of maternal abuse yet it is still puzzling why mothers sometimes abuse their infants. Females ranked highly in the dominance hierarchy tend to use a more 'laissez-faire' maternal style while lower ranking mothers are more protective of their offspring in larger groups, demonstrating an individual variation in maternal styles (Altmann, 1981; Suomi 2005), but they still exhibit similar rates of abuse than middle ranking female mothers (Sanchez et al. 2010). If a mother is high ranking and abusive, why would the mother also exhibit controlling behaviors when it is not necessary? While maternal abuse is most likely a learned behavior,

the majority of mothers do not abuse their infants and could also provide models of positive rearing.

This study examines whether differences in behavioral inhibition occur between abused and control infants and may shed additional light on the negative effects of maternal abuse. Rates of exploration versus behavioral inhibition (reduced exploration, slowed motor activity, higher fear and anxiety-like behaviors), use of the mother as a secure base from which to explore, attachment, and stress vocalizations were used to determine the behavioral inhibition and secure base behavior of infants. In addition to the effect of differential maternal care experienced early in life, I also examined its effect on male versus female infants.

Methods and Materials

Subjects

This study was conducted using infant rhesus macaques (*Macaca mulata*) living in social groups at the Yerkes National Primate Center Research Center Field Station, in Lawrenceville, GA. Study subjects included 33 six month old infant macaques who were cross-fostered within 24 hours of birth (except for one cross-foster, which was performed within 72 hours), to a "control" (n=17, 9 female & 8 male infants) or "maltreating" (n=16, 6 female, 10 male infants) mother, following a semi-random design.

There were four categories of cross-foster: control-to-abused, abused-to-control, control-to-control, and abused-to-abused (Table 2). Females with a history of abusive behavior were closely monitored and ultrasounds were performed to estimate their date of parturition. Once two females gave birth, ideally within 48 hours, a cross-foster procedure was planned. Both mothers would be captured and put into squeeze cages to gently remove the infant from the mother. The infant would then be wrapped in a blanket and transported quickly to the other compound. The infant would then be given to the foster mother and if the foster infant was immediately picked up and put on ventrum, she would be transferred into an indoor housing area (capture unit). Then the mother and infant would be monitored between 30 minutes to 4 hours to ensure that the mother was holding the infant and allowing it to go on nipple to nurse before being released into their group. Once in the group, the mother-infant pair would be monitored daily for one week to ensure a successful cross-foster. This study had a 78% success rate for infant cross-fosters.

The subjects lived in four different social groups and were housed in outdoor compounds with access to indoor housing with climate control for extreme weather

conditions. All groups had a stable matrilineal structure and a linear dominance hierarchy. Female dominance ranks were determined from observations of aggressive and submissive behaviors, and determined from previous studies. Food and water were provided *ad libitum*.

Yerkes Colony Management and the Sanchez lab monitored the subjects to ensure the well-being of the infants. In cases where physical abuse poses a threat to the physical integrity of the infant the Veterinary and Animal Care departments intervenes and in severe cases remove the infant from the mother and raise it in the Yerkes National Primate Center nursery. However, to prevent this issue, all females who have a history of causing significant injuries to their infants have been removed from the breeding compound or placed on contraceptives (DepoProvera).

Table 1: Center Emory Subjects:

Control-to-Abused Cross-foster

Focal	Name	DOB	Sex	Foster	Foster group	Rank
Fv13	Fiver	4/14/2010	M	Ln10	BC2A	High
Py13	Pudgy	4/27/2010	M	Eh9	BC2A	Low
Ub14	Ugbug	5/15/2010	F	Vt10	A2	Middle
Lc14	Liberace	5/19/2010	M	Pa10	BC2A	Low
Vf14	"Vader"	6/16/2010	M	Wo3	A2	Middle
Ch13	Charlie	4/23/2009	F	Cf10	T3	High
Im113	Imani	5/20/2009	F	Mo8	T3	Middle
Rm13	Raymus	5/23/2009	M	Ld9	T3	Middle

Abused-to-Control Cross-foster

Focal	Name	DOB	Sex	Foster	Foster group	Rank
Sb14	Sombrero	5/15/10	M	Hn10	A1	High
Nc14	Nancy	5/19/10	F	Dj7	A2	Middle
Uf14	Umbelliferous	6/16/10	F	Mv5	A1	Low
On14	Oblong	4/26/2011	F	Bp10	A1	Middle
Bf13	Butterfly	4/15/2009	F	Iq9	T3	High
Hm13	Homer	5/20/2009	M	Hg6	BC1A	High
Tm13	Thumper	5/23/2009	F	Ls5	BC2A	Middle

Control-to-Control Cross-foster

Focal	Name	DOB	Sex	Foster	Foster group	Rank
Me14	Melvin	6/3/10	M	Nl7	BC2A	Middle
Ne14	Nemo	6/3/10	M	Hw4	A2	High
Ym14	Yuma	4/20/2011	M	Gq10	A1	Low
Wm14	Wham	4/20/2011	F	Kj8	BC2A	Low
Bo14	Boris	5/2/2011	M	Du7	BC2A	Low
Fo14	Fork	5/3/2011	M	Pl8	A1	Low
Nt14	Nitro	6/7/2011	M	Bu5	BC2A	Low
St14	Sprite	6/10/2011	F	Fi9	A1	Middle
Zu14	Zuul	6/22/2011	F	Ai10	BC2A	High
Me14	Merlin	4/13/2009	M	Uy8	A1	High
Je13	Jem	4/13/2009	F	Wm3	BC2A	Middle

Abused-to-Abused Cross-foster

Focal	Name	DOB	Sex	Foster	Foster group	Rank
Pv13	Percival	4/17/10	M	Ck7	A1	High
Rv13	Ravioli	4/17/10	F	Lo9	BC2A	High
Dw13	Dwayne	4/19/10	M	Rm7	A1	Middle
Ew13	Erwin	4/19/10	M	En10	BC2A	Middle
Kr14	Kougar	5/22/2011	M	Le9	A1	Middle
Ws14	Witherspoon	6/1/2011	F	Re10	A2	Low
Ul13	Umali	5/17/2009	F	Me6	A1	Middle
Yl13	Yentl	5/18/2009	M	Eq4	BC2A	Middle

Table 2: Compound Summaries

2009 & 2010 Compound Summary

Compound	Total Members	Adult Males	Adult Females (reproductive age: >4 years of age)	Main Matrilines
A1	No recent Data*	6-8	approximately 100	6
A2	No recent Data*	2	60-70	9
BC2A	No recent Data*	3-5	approximately 40	7
BC1A	No recent Data*	2-3	approximately 45	2

*No detailed information was kept for 2009-2010. The total number of group members was higher prior to 2011.

2011 Compound summary

Compound	Total Members	Adult Males	Adult Females (reproductive age: >4 years of age)	Main Matrilines
A1	164	6	84	6
A2	87	2	38	5
BC2A	99	5	41	7

Procedures

In order to examine the effects of infant maltreatment and gender on the infant's behavioral reactivity to novelty, including behavioral inhibition and secure base behavior, the Laboratory Temperament Assessment Battery (Lab-TAB) task, developed by Dr. Judy Cameron at the Oregon National Primate Research Center (Bethea et al. 2004; Cameron et al. 2003; Williamson et al. 2003), was used. This test was adapted by Dr. Cameron for use with infant rhesus monkeys (3-12 months old) from the Lab-TAB originally developed for human children (Goldsmith & Rothbart 1991). The rhesus infant Lab-TAB is divided into four consecutive tests: Free Play (35 min in a novel room with toys), remote controlled car (RCC), (2 min with a RCC being driven towards the infant), the Human Intruder test (15 min, with the mother being in an adjacent cage with a human standing in front of the infant cage, either presenting his/her profile to or making direct eye contact with the infant) and a novel fruit presentation (presentation of a reward behind a threatening stimulus). For this study, only the first test was focused on: Free Play test.

The testing room was 2.3m long x 2.85m wide with two one-way windows for videotaping and monitoring. A climbing structure (150 cm long X 125cm tall X 30 cm wide) was located opposite to the door and there were novel toys (9) arranged in a semicircle (see Image 1). Mother and infant were placed together in the test room. The floor was marked in a 1 sq foot grid to assist in measuring infant distance from the mother. There was a camera inside the testing room and another one behind a one-way mirror to capture different views during testing.

Both mother and infant were removed from their group in the morning and placed into a squeeze cage. The mother was anesthetized (6 mg telazol/kg BW, i.m.) to avoid the confounding effect of her behavior on the infant's behavior. Both individuals were quickly transferred to the Lab-TAB room where the mother was placed in a car seat with a waterproof pad; the infant typically stayed on the mother's ventrum. Videotaping began once the experimenters left the Lab-TAB room and locked the access door. Both the mother and infant were monitored constantly through the one-way mirror. On the rare occasion when the mother started to come out of the anesthesia during the test, the test was paused, a ketamine supplement was given, and the mother was restored to a comfortable seated position.

Observations lasted 35 minutes. Experimenters recorded the initial latency for the infant to leave the mother (all 4 limbs on the ground or climber). The infant was then free to explore the unfamiliar environment with novel toys while the mother was sedated in the car seat.

Behavioral Scoring

Videotapes were coded using the two synchronized camera views (inside and outside of the room) for the behaviors included in the Lab-TAB ethogram (see Table 3). I coded all behaviors in the ethogram, including exploration, locomotion, anxiety-like behaviors, interactions with mother, and vocalizations, remaining blind to group assignment. Exploration was broken down into visual room exploration, physical exploration of objects, and physical exploration of the car seat. Frequencies and durations of the behaviors were corrected for total test duration, and excluded during periods where there was an outside disturbances or the infant was out of view. The videos were edited on Adobe Premiere Pro and coded using The Observer XT 10 (Noldus Information Technologies Inc, Leesburg, Virginia).

Before I began coding these tapes, I was trained to achieve intraobserver and interobserver reliabilities of ≥80% for frequency and duration behaviors. Training consisted of real-time and videotape scoring of observed behaviors.

Statistical Analysis

The effects of maternal care and infant sex on behavior during the Free Play test were analyzed using SPSS version 19.0. For low occurrence behavioral categories (behaviors that occurred in less than half of the sessions), the behavior was transformed from durations and frequencies to categorical data ("yes" or "no") based on whether or not each animal performed the behavior. Thus, groom, away from mother, locomotion, manipulate mom, explore object, and sleep were transformed to categorical data, while all other behaviors were analyzed as frequencies and durations. The categorical data was analyzed using Chi-square for Two-way (or contingency) tables and reported as Pearson

Chi-square statistic for group effects. All other duration and frequency data with normal distributions (or log-transformed non-normal distributions) were analyzed using Two-Way ANOVA, with maternal care (control vs. maltreated) and sex (male vs. female) as fixed factors. Behavioral data that violated the assumption of normality and homogeneity of variances even after transformation were analyzed using nonparametric Friedman Two-Way analysis of variance. Significance levels were set at $p<0.05$ for all analyses.

Results

In this study, abused infants spent significantly more time on their mother's ventrum than did controls ($F_{1,29}$=4.809, p=0.036, Figure 1). No significant differences were found between males and females ($F_{1,29}$=0.372, p=0.547), nor was there any interaction effect of Gender and Foster condition on time spent on the mother's ventrum ($F_{1,29}$=0.012, p=0.912).

There was a trend for control infants to spend a greater percentage of time cooing than abused infants ($F_{1,29}$=2.254, p=0.144). No significant differences were found between males and females ($F_{1,29}$=1.515, p=0.228). There was no interaction effect of Gender and Foster condition on time spent cooing ($F_{1,29}$=0.631, p=0.434).

Female infants spent significantly more time exploring the car seat than did males ($F_{1,29}$=5.217, p=0.030). No significant differences were found between Foster conditions ($F_{1,29}$=1.525, p=0.227), nor was there any interaction effect of Gender and Foster condition on time spent exploring the carseat ($F_{1,29}$=0.117, p=0.735).

There was a trend for abused infants to spend a greater percentage of time exploring the room than control infants ($F_{1,29}$=2.940, p=0.097). However, no significant differences were found between males and females ($F_{1,29}$=.049, p=0.827), as well as no interaction effect of Gender and Foster condition on time spent exploring the room ($F_{1,29}$=0.001, p=0.982).

Control infants spent significantly more time exploring objects than did abused infants ($F_{1,29}$=4.707, p=0.039). There was also a trend for interaction effect of Gender and Foster condition on time spent exploring objects ($F_{1,29}$=2.823, p=0.104). However, no significant differences were found between males and females ($F_{1,29}$=1.271, p=0.269).

No additional main or interaction effects reached significance for Foster group or Gender on the remainder of the behaviors analyzed in this study. Rank was also analyzed for behaviors with normal distributions using Two-Way ANOVA, with maternal care (control vs. maltreated) as fixed factors, but no significant effects were found across all behaviors.

Figure Legends

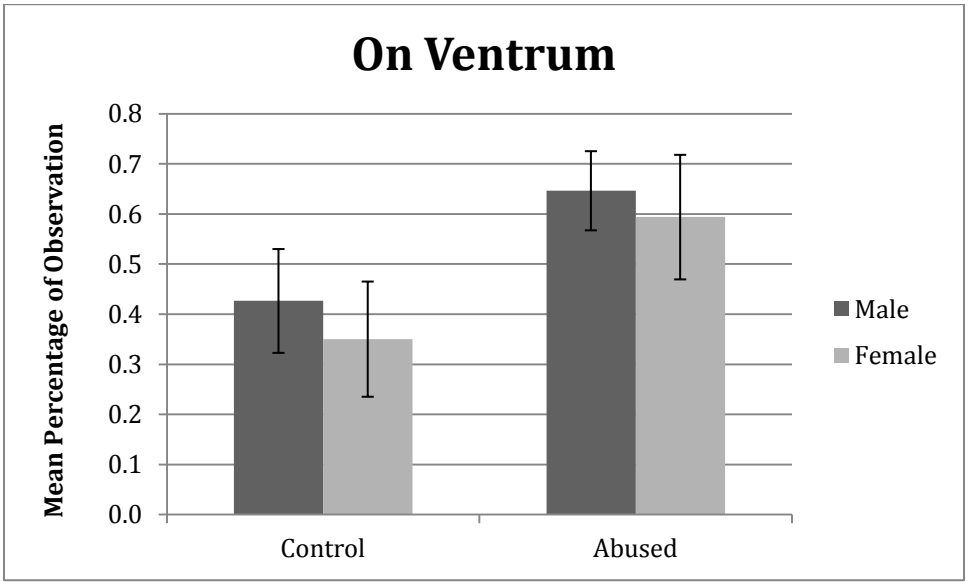


Figure 1. Time spent "on ventrum". A significant main effect of maternal care (Foster mom factor) was detected ($F_{1,29}$=4.809, p=0.036), with abused animals spending a higher percent of time on ventrum than control animals. No main Gender ($F_{1,29}$=0.372, p=0.547) or Foster x Gender interaction effects ($F_{1,29}$=0.012, p=0.912) were found.

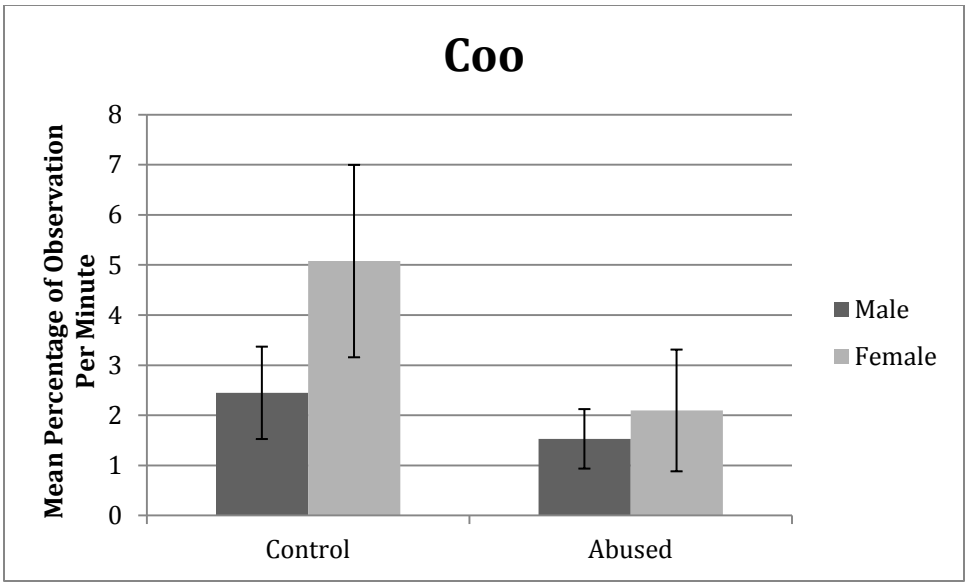


Figure 2. Coo vocalizations. No significant main effect of Foster group was detected ($F_{1,29}$=2.254, p=0.144), despite a trend for control animals to spending a greater percentage of time cooing than abused animals. No main Gender ($F_{1,29}$=1.515, p=0.228) or Foster x Gender interaction effects ($F_{1,29}$=0.631, p=0.434) were found either, although female controls seem to coo more than female abused infants.

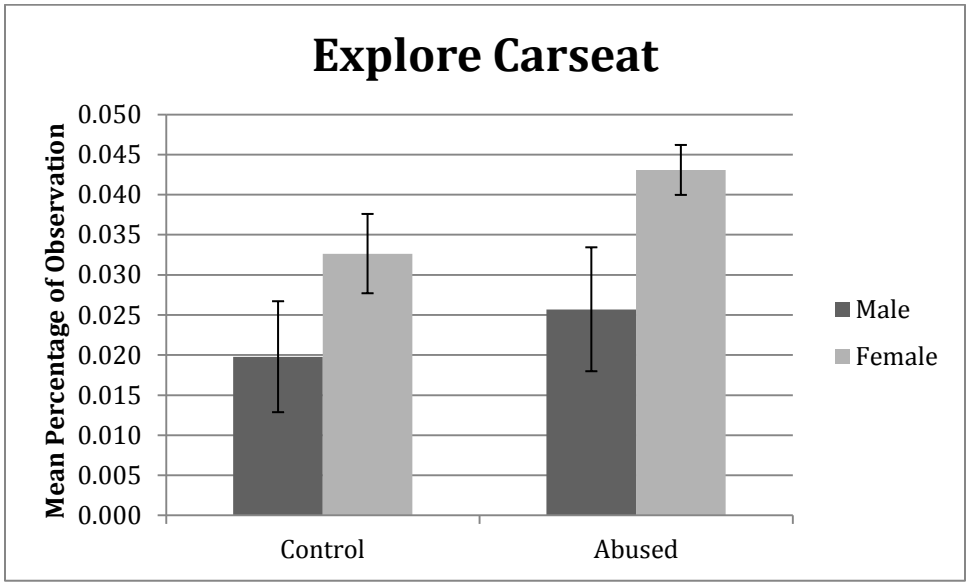


Figure 3. Time spent exploring the carseat. A significant main effect of Gender was detected ($F_{1,29}$=5.217, p=0.030), with females spending a greater percentage of time exploring the carseat than males. No main Foster Mother ($F_{1,29}$=1.525, p=0.227) or Foster x Gender interaction effects ($F_{1,29}$=0.117, p=0.735) were found.

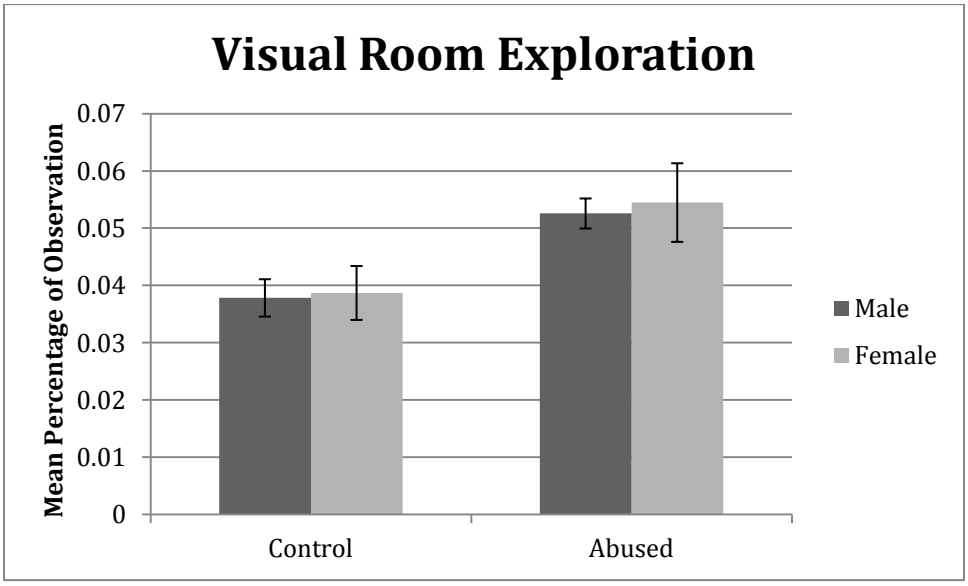


Figure 4. Time spent exploring the room. A trend of maternal care (Foster mom factor) was detected ($F_{1,29}$=2.940, p=0.097), with abused animals spending a greater percentage of time exploring the room than controls. No main Gender ($F_{1,29}$=.049, p=0.827) or Foster x Gender interaction effects ($F_{1,29}$=0.001, p=0.982) were found.

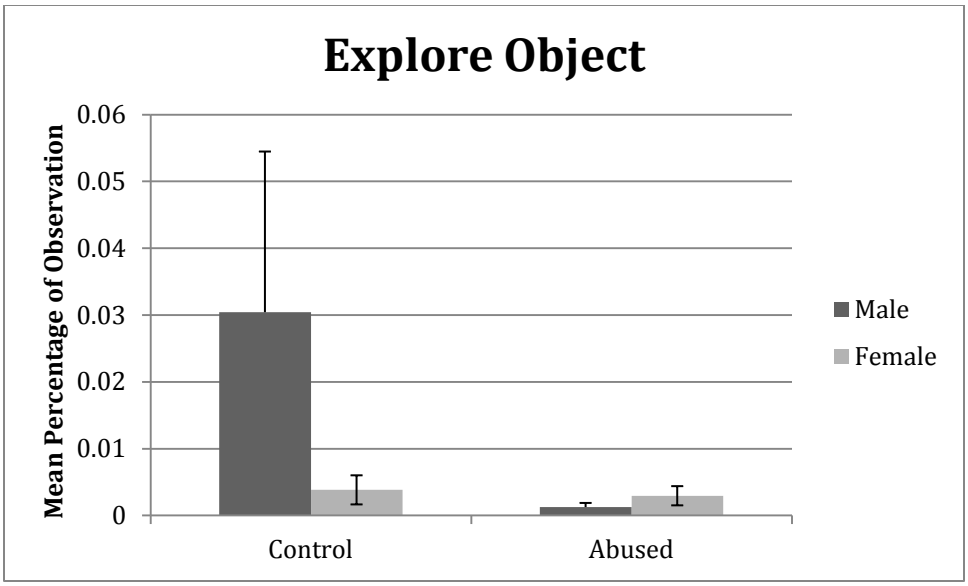


Figure 5. Time spent exploring objects. A main significant effect of maternal care (Foster mom factor) was detected ($F_{1,29}$=4.707, p=0.039), with control animals spending a greater percentage of time exploring objects than abused infants. No main Gender effects ($F_{1,29}$=1.271, p=0.269), or Foster x Gender interaction effects ($F_{1,29}$=2.823, p=0.104) were found.

Discussion

In this study, maltreated infants exhibited higher behavioral inhibition (stay in close contact with the mother) and explored objects away from the mother less than did controls during the Free Play laboratory task. The results also provided interesting findings of gender differences in exploration. The hypothesis that abused infants would be more fearful (behaviorally inhibited) in a novel environment and, therefore, would spend more time in close proximity to the mother, and would explore less than controls was supported by the results. Additionally, explorations that primarily occurred in contact with the mother, exploring the room and the car seat, were performed at higher rates by abused infants than by controls. The gender differences detected, with females emitting more (coo) vocalizations and exploring the space in close proximity to the mother (the car seat) more than males, whereas males spent more time exploring objects, suggests that males might be less inhibited and more risk-taking.

Abused infants spent significantly more time on ventrum than control infants. For a mother to be assigned to the maltreatment group, she must have been observed both abusing and rejecting an infant within the first two weeks of its life. During group observations, McCormack et al. (2006) found that abusive mothers had higher rates of infant rejection and broke the contact more frequently than did the mothers of control infants, especially during the first three months of an infant's life. Abused infants, in turn, broke contact with their mothers less often than controls, suggesting delayed independence and an insecure attachment. Abused infants are also less likely to break contact with the mother because they are more likely to be forced off by the mother or rejected (McCormack et al. 2006; Maestriperi & Carroll 1998). Under these test conditions,

infants were able to choose how much time to spend on the mother and could freely keep ventral contact with the mother and remain on the nipple. During the Lab-TAB test, with the mother sedated, infants were able to freely explore the novel room while the mother acted as a safe base. Outside of these conditions, the abusive mother is more likely to reject the infant so it is not surprising that abused infants spent more time in close contact with the mother on ventrum, consistent with previous reports of delayed independence from the mother (McCormack et al. 2006). It is important for infants to form a secure relationship with the mother. These findings are in agreement with reports that children who have insecure connections with their mothers are also less likely to explore novel environments (Shonkoff 2005). During separation experiments with children, abused children showed lower levels of stress hormones evaluations and behavioral signs of being upset, and when the mother was reintroduced, the infant did not immediately seek proximity (Morton & Browne 1998). When interacting in the world, a child's insecurity can also affect their ability to meet new friends and form new relationships (Crittenden 1986). This insecurity would seem to be maladaptive because it could hinder the child's adjustment to new situations later in life and also negatively influence the attachment to his or her own child.

One question that has arisen is why do infants that are being abused still want to be in close contact with the mother? At the age of six months, it is believed the brain circuits responsible for fear and avoidance learning are not as developed because infants are still in a sensitive period where it is important to be near to the mother (Helfer et al. 1997). This means that at the age of six months, rhesus infants have been "selected" to ensure that there is a strong bond with the caregiver despite repeated abuse or neglect. This has also

been found to be true in humans around four years old (Helfer et al. 1997). Infants form a bond despite the abuse that they may receive because they need to be protected in the first months of life. Moriceau & Sullivan (2005) hypothesized that rats may have developed this close bond to prevent pups from learning aversions to the mother, so that they can still receive milk, warmth, and protection, despite rough handling in the nest or shock-induced learning in a lab.

One result in the present study was a trend that maltreated infants visually explored the room more than control infants, but only when in close physical contact with the mother. An important distinction is that exploring the room was a form of visual exploration and did not involve leaving the mother and they often appeared to be anxious when visually exploring. Exploration of the room was also especially common in the beginning of the test as the infant adjusted to the novel room, and could be interpreted as a "vigilant" behavior (Rogers et al. 2008). Infants with insecure attachments are often characterized as being more anxious and so it would be expected that the infant might visually explore the room more when put into a novel situation (Crittenden & Ainsworth 1989). The infant still retains the safe base of the mother while being free to visually inspect the room. A control infant may be less stressed by the novel situation and able to overcome its anxiety to leave the mother's secure base and explore new objects (Crittenden & Ainsworth 1989).

Control infants were found to exhibit higher durations of exploring objects compared to abused infants. To explore objects, the infant must break contact with the mother and locomote to the objects (which is an approach behavior, more likely motivated by the intrinsic curiosity in the species) (Ryan & Deci 2000; Mears & Harlow 1975).

Therefore, exploring objects shows a more directed effort and costly form of exploration, leaving the safe base. Due to the control infant's secure attachment to its mother, the infant is more likely to explore new environments because the mother has reliably acted as a safe base in the past, fitting well with typical "refueling behavior" as reported in the literature (Hinde & Spencer-Booth 1967; Vyt 1989).

Though not statistically significantly different, control animals also showed a trend to coo more than abused infants. Coo vocalizations are commonly emitted to call out for help or call the mother's attention in a time of distress. In this test, mothers were sedated and unable to respond to vocalizations. In the compounds, control infant's mothers generally come to the aid of their infants (Hammerschmidt et al. 2001). A control infant is used to the mother being supportive and responsive; so when the mother is not reacting in the way she typically does, the control infant may become more anxious, which leads to higher rates of cooing (Tomaszychki et al. 2001). Abused infants, perhaps not expecting their mother to always aid them, did not coo as often. Similar results of increased control infant vocalizations (screams, in that case) have been shown in human intruder tests where the mother is kept in a separate cage and cannot retrieve the infant (Howell et al., unpublished data). In the Human Intruder test, a threatening stimulus is present, causing distress, including distress calls which cannot be responded to. Human children are particularly vulnerable because they cannot walk until approximately one year of age. Without locomotion, children can only gain a caregiver's attention by producing distress calls. Human children display behaviors such as smiling and crying that are intended to attract the caregiver (Morton & Browne 1998). When the usual behaviors that attract the mother do not produce a response, the infant may experience higher levels of distress.

After reviewing the data, subjects that cooed more than 200 times all had high rates of being away from the mother. Infants that left the secure base of the mother most likely had higher levels of anxiety, leading to more calls to the mother compared to infants who spent the majority of their time in close proximity (Crittenden & Ainsworth 1989). Infants were able to leave the mother and explore but were still distressed and produced vocalizations. While other studies have found sex differences for coo vocalizations (Tomaszycki et al. 2001; Lovejoy & Wallen 1988) with females vocalizing more than males, no significant differences were found in this study.

In this study, males showed a tendency toward increased exploration of objects, though this result was not significant. Males may have explored objects more since young females generally spend more time in proximity with the mother and so leaving the mother might have been more distressing for females in comparison to males (Lovejoy & Wallen 1988). In the wild, male infants have been found to leave the mother sooner than females (Mitchel 1968). For males it is important to be exploratory and more independent because they will have to leave the troop once they reach puberty (Mitchel 1968). Male infants interact more with other group members outside of their matriline and are more likely to solicit play from other infants (Mitchel 1968; DiPietro 1981; Bernstein et al. 1993). In particular, males participate more in rough and tumble play than females. This type of play is one of the primary ways males learn normal social development (Wallen 1996).

Studies have also been performed in rhesus and humans to determine sex differences in toy preference. In humans, sex preferences were present at nine months, with toy coloring and representation possibly affecting preference (Alexander et al. 2009). Males have been found to have more rigid preferences for toys compared to females so it

may be that males were more drawn to the toys that were selected for this task due to varying proximities of the objects and differing shapes and colors (Jadva et al. 2010).

One of the more surprising results was that females explored the car seat more often than males. Although, it is difficult to say why females may have explored the car seat more, but it may relate to the way most infants explored the car seat. Exploration of the car seat took place while in proximity to the mother. Therefore, the mother was still providing a safe base from which the infant could explore the novel room. Females explored more while in close proximity to the mother, which suggests that females were more cautious or that they are more behaviorally inhibited. Lovejoy and Wallen (1988) found that female infants spent more time in proximity with mothers and were more distressed than males when they were separated. This avoidance strategy by females of ignoring exploration to cope with fear and novelty may actually reinforce the behavioral inhibition and can later lead to continued behavioral inhibition and social anxiety (Degnan & Fox 2007). Male infants were willing to take higher risks by exploring off of the mother and thus overcoming their behavioral inhibition.

Although some of these behaviors are comparable across rhesus and human infants, there are limitations when trying to translate the interpretations of these results to humans. While rhesus monkeys serve as a good model organism to test early effects of early experience on human infant attachment, the developmental rates for the two species are very different. In general, rhesus develop three to four times faster than human children (Francis et al. 2008). Most studies examining human child maltreatment looked at children who had experienced maltreatment before the age of five years old while this study looked at six month old subjects (equivalent to a two year old human). Researchers

have yet to define the sensitive periods in primates, as well as other inter-species differences, related to socialization outcomes (O'Connor & Cameron 2006). Human behavior is also much more complex and infants might be attracted to different stimuli during the test.

When looking at these results it is also important to remember that the free play section of the Lab-TAB is only one part of the test. This test was performed to determine maternal attachment, exploration, behavioral inhibition, anxiety, and fear of the infant, as well as its temperament. The test also examined how the infant interacts with the mother when she is not awake to restrain the infant or care for it. The infant is alone in the sense that it is comforted by the safety the sedated mother presents but is also in a novel room with new objects and structures it is not used to seeing or interacting with. The other parts of the Lab-TAB can provide a more complete analysis of anxious behavior under different contexts: with or without the presence of a threatening stimulus or a social versus non-social threat situation (Cameron et al. 2003). When put together, these results can help reveal how maltreated and control infants perform on different tasks and thus elucidate behavioral differences that are present early in life.

Though this experiment was well executed, there were a few factors that might have affected the results. One challenge we encountered in running behavioral experiments is that there is, inevitably, a limited sample size and therefore statistically significant differences or trends are less likely in a small population. However, given the number of monkeys at Yerkes and the requirements to be classified as an abusive mother, ours is a big sample size for studies with rhesus monkeys and it is not realistic to expect a larger sample size for very intensive assessments, which also include correlates to brain development.

Also, given the sample size, there was not an even distribution of maternal rank for maltreated and control infants. For instance, there were ten abused middle ranking infants but only three high and three low ranking abused infants. To ensure group stability in the matrilines, very high ranking females are usually not taken out of the group for tests because doing so could lead to rank challenges and, possibly, an overthrow. Therefore, an even distribution of ranks among subjects is difficult to ensure given colony management procedures and the availability of pregnant mothers.

Discovering the factors influencing infant maltreatment in nonhuman primates can help researchers better understand at-risk individuals in human populations and ways to minimize later adverse effects. Future research could benefit from having larger sample sizes and a more balanced rank distribution among subjects. In general, more studies need to be performed looking at the behavioral differences between maltreated and control infants, particularly longitudinal studies that might better demonstrate the long term effects and consequences of abuse that may affect humans. This Lab-TAB test allows researchers to investigate what behavioral differences arise early in life and how early maltreatment alters exploration and mother-infant attachment.

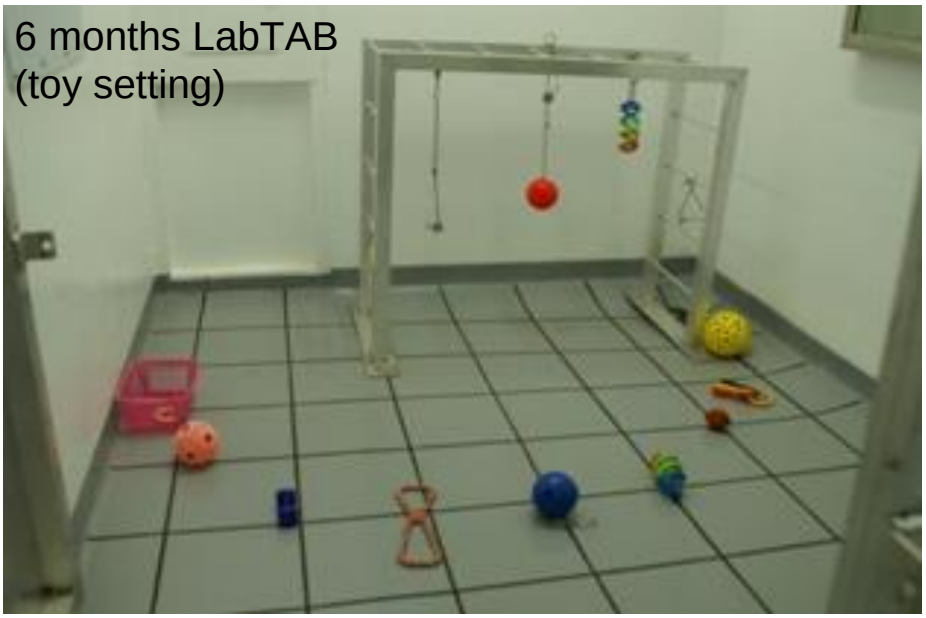
Conclusion

In summary, the present study supports the initial hypothesis that maltreated infants are more likely to stay close to the mother and to explore less and emit more distress calls than controls. While studies on gender differences in early infant behavior are still growing (Jadva et al. 2010), these results may show that different genders express behaviors at different times during development. This study showed that maltreated infants are more likely to explore a car seat while in contact with the mother than to explore distant objects. Control animals also cooed more than maltreated infants, most likely due to the inability of the sedated mother to respond to the infant's calls.

These findings are valuable because they suggest that there are early behavioral differences between maltreated and control infants. These findings might have implications for children who were maltreated or were raised in poor orphanage conditions because differences in exploration and proximity were discovered between control and maltreated infants regardless of the biological mother's parenting style.

Appendix

Image 1: Testing Room



This photo is the property of the Yerkes National Primate Center. This photo is confidential and not for distribution.

Table 3: Lab-TAB Free Play Ethogram

CODE	BEHAVIOR	DESCRIPTION OF BEHAVIOR		
	Exploration (room, seat, objects):			
ex	explore	enter <u>modifiers</u> (i.e. receivers):		
		ro	room	[ev]: looking around the playroom (> 3 sec)
		ca	car	[ev]: looking at the car –not car seat- (> 3 sec)
		cs	car seat	[ce]: includes pad; visual, oral or tactile investigation of seat (> 3 sec)

ob object [oe]: inspect, sniff, touch or manipulate object (not slap or bite)

e- explore end ends explore behavior; if explore type changes, the new type ends the previous (e.g. ex, ro, ca rather than ex ro, e-, ex ca).

ac *approach (car) subject moves towards car

Activity:

lo locomotion self-induced movement that results in change of location; includes walking, bouncing, running, jumping & dropping from structures/ceiling. [Off mom/seat].

l- locomote end [locomote and climb may end each other]

cl climb (vertical movement) [ceiling, windows, camera –not car seat-]
Enter <u>modifiers</u>:
- cb on climber
- os on other structures

c- climb end [locomote and climb may end each other]

sl sleep inactive, with eyes closed (>3sec)

s- end sleep

pa passive/stationary Inactive (still and calm); not explore/sleep, or other behavior (> 3sec)

p- end passive

Aggressive/dominance:

ag *contact aggression includes bite, hit & slap.
Enter <u>modifiers</u>:
- ca car
- cs car seat
- ob object
- mo mother
- se self
- ot other

th *threat (noncontact aggres.) open mouth stare (w/ or w/o vocalization); includes head bobb and lunge
(quick forward movement towards object); eyes wide open; code also
"raised eyebrow" (stare w/o open mouth)]. Without contact.
Enter <u>modifiers</u>:

ca	car
cs	car seat
ob	object
mo	mother
se	self
ot	other

ct crooked tail Enter <u>modifiers</u>:

ca	car
cs	car seat
ob	object
mo	mother
se	self
ot	other

ce end crooked tail (mirror)
di display subject bounces up & down, shaking/biting equipment & objects
d- end display

Submissive:
li *lipsmack repeated lips movements, pressing them together with soft
clucking/smacking noises; ears pulled back. Counted as "bouts".
Enter <u>modifiers</u>:

ca	car
cs	car seat
ob	object
mo	mother
se	self
ot	other

pr *present orienting hindquarters towards stimulus with raised tail.

Enter modifiers:

ca	car
cs	car seat
ob	object
mo	mother
se	self
ot	other

Displacement/self-directed:

ss *Scratch subject uses hands or feet to scratch an area of body; usually rapid strokes. New bout after 3 seconds

ya *Yawn common definition

bs *body shake shaking head and body like a wet dog.

sg self-groom picking and spreading own fur using hands, feet or mouth

se end groom ends both self-groom and groom

Fearful/defensive:

fr freeze motionless, except for slow head movements; tense body posture; hanging or on ground (> 3 seconds).

f- end freeze

gr *fear grimace lips pulled back exposing clenched teeth; ears pulled back.

Enter modifiers:

ca	car
cs	car seat
ob	object
mo	mother
se	self
ot	other

es *escape (car) infant runs away from the car

wd *withdrawal quick, jerky motion backwards, away from car/object

ta turn away infant turns its back towards car/room, not looking; usually facing mother's ventrum. [If in response to "noise outside", mark this as an event and then edit out (do not code)].

t-	end turn away	
av	avert (visual avoidance)	facing the car/object with tense body posture, the subject makes quick shifts in gaze, avoiding eye contact.
a-	end avert	
sc	*scream/screech/shriek	distress, high pitch, vocalizations
oo	*coo	soft "call" vocalization made by rounding and pursing the lips; medium pitch and intensity. [If can't be heard, check inside camera; if after that still can't be heard, code if can be seen]. Scored in bouts if occurring consecutively with high frequency (1 coo scored per 3 heard in this case)
tt	*tantrum	infant's body shakes, while geckering or screaming
ud	*urination/defecation	common definitions

Mother-infant interaction:
NOTE: co, px and aw are MUTUALLY EXCLUSIVE and must always be scored

co	contact	infant initiates physical contact with mother (any type of contact)
px	proximity	animal enters and stays within mother's arm length (1 ft); can be out of view. [If infant is on car seat…it is in proximity to mom]
aw	away	infant is not in contact or prox (breaks physical contact with mother)
vo	on *ventrum*	infant's torso (ventral or dorsal) is in contact with mom's ventrum. Contact must be scored before ventrum.
v-	off *ventrum*	ventral contact ends
no	on nipple	
n-	end on nipple	
gm	groom	infant picks and spreads mom's fur with hands/teeth/tongue, checks her mouth, arms, etc
g-	end groom	
mb	manipulate mom's body	(e.g. checking her mouth/arms, pushing mom from behind)
m-	end manipulation	
cm	*Comfort	infant returns to mother when car advances

Other:

ov	out of view	common definition
o-	end out of view	
lr	looking at researchers through the window	
le	end looking at researchers	
dp	depressive-like	head down, self-clutching, rocking
de	end depressive-like behavior	
at	atypical	species-atypical behaviors, including stereotypic behavior (repetitive motor pattern –e.g., circling, pacing, jumping- that occurs 3 or more consecutive times), bizarre posture, etc
ae	end atypical behavior	
er	erection	
ee	end erection	
ma	masturbation	
me	end masturbation	
od	*outside disturbance	

NOTES:

- *= frequencies (behaviors without * are scored as durations)
- Coo should be coded if not heard but can be seen in tape (inside tape should help with confirmation)
- Add comments (e.g. behaviors not in the above ethogram) and mark unusual events such as “noise/disturbance outside” (so that we can go back in the tape and eliminate the behaviors displayed in response to that event.

References

Aksan, N. & Kochanska, G. 2004. Links between systems of inhibition from infancy to preschool years. *Society for Research in Child Development* 75: 1477-1590.

Alexander, GM, Wilcox, T., & Woods, R. 2009. Sex differences in infants' visual interest in toys. *Animal Sex Behavior* 38: 427-433.

Allen, RE & Oliver, JM. 1982. The effects of child maltreatment on language development. *Child abuse and Neglect* 6:299-305.

Altmann, JA 1962. A field Study of the Sociobiology of Rhesus Monkeys, *Macaca mulatta*. *Annals of the New York Academy of Sciences* 102: 338-435.

Altmann, J. 1981. *Baboon mothers and infants*. Boston: Harvard University Press.

Bernstein, IS 1970. Primate status hierarchies. In L.A. Rosenblum (Ed.), *Primate Behavior: developments in field and laboratory research* 1: 71-109. New York: Basic Books.

Bernstein, IS, Judge, PG, & Ruehlmann, TE. 1993. Sex differences in adolescent rhesus monkey behavior. *American Journal of Primatology* 31: 197-210.

Bethea CL, Streicher JM, Coleman K, Pau FKY, Moessner R, Cameron JL. 2004. Anxious behavior and fenfluramine-induced prolactin secretion in young rhesus macaques with different alleles of the serotonin reuptake transporter polymorphism (5-HTTLPR). *Behavior Genetics* 34: 295-307.

Bowlby, J. 1969. *Attachment and Loss*. New York: Basic Books.

Bowlby, J. 1977. The making and breaking of affectional bonds. *The Royal College of Psychiatry* 130: 201-210.

Brent, L., & Koban, T. 2002. Abnormal, Abusive, and Stress-Related Behaviors in Baboon Mothers. *The society of Biological Psychiatry* 52: 1047-1056.

Calkins, SD, Fox, NA. 1992. The relation among infant temperament, security of attachment, and behavioral inhibition at 24 months. *Child Development* 63(6): 1456-1472.

Cameron, JL., Bacanu, SA., Coleman, K., Dahl RE., Devlin BJ., Rogers, J. Ryan, N., Williamson, D. 2003. Dissociating components of anxious behavior in young rhesus monkeys: A precursor to genetic studies. In Gorman JM, (ed.) *Fear & Anxiety: Benefits of Translational Research.* Washington DC: American Psychiatric Press, pp 251-271.

Cameron, J. 2004. Fear and anxiety: The benefits of translational research. *American Psychiatric Publishing*, Inc., ed.1: 211-222.

Campbell, C., Fuentes, A., MacKinnon, K., Bearder, S., Stumpf, R. 2011. *Primates in Perspective*. New York: Oxford University Press.

Champagne, FA & Curley, JP. 2009. Epigenetic mechanisms mediating the long-term effects of maternal care on development. *Neuroscience and Behavioral Reviews* 33: 593-600.

Coplan, JD, Andrews, MW, Rosenblum, LA, Owens, MJ, Friedman, S., Gorman, JM, & Nemeroff, CB. 1996. Persistent elevations of cerebrospinal fluid concentrations of corticotrophin-releasing factor in adult nonhuman primates exposed to early-life stressors: Implications for the pathophysiology of mood and

anxiety disorders. 1996. *Proceedings of the National Academy of Sciences* 93: 1619-1623.

Crittenden, PM 1986. Final Project Report. HHS Grant #90-CA-844: Differential assessment of maltreating families using interaction, attachment and family systems.

Crittenden, PM & Ainsworth, M. 1989. Child maltreatment and attachment theory. In Cicchetti, D. & Carlson, V. (Ed.) *Child Maltreatment*: 432-463. New York: Cambridge University Press.

Degnan, KA & Fox, NA. 2007. Behavioral inhibition and anxiety disorders: Multiple levels of a resilience process. *Development and Psychopathology* 19: 729-746.

DiPietro, JA. 1981. Rough and tumble play: a function of gender. *Developmental Psychology* 17(1): 50-58.

Egeland, B. 1991. A longitudinal study of high risk families: issues and findings. In Starr, R. & Wolfe, D. (eds.), *The effects of child abuse and neglect: Issues and research* pp. 33-56. London: Guilford Press.

Essex, MJ, Klein, MH, Cho, E., & Kalin, N. 2002. Maternal stress beginning in infancy may sensitize children to later stress exposure: Effects on cortisol and behavior. *Society of Biological Psychiatry* 52: 776-784.

Fox, NA 2004. Temperament and early experience form social behavior. *Annals New York Academy of Sciences* 1038:171-178.

Francis, P., Appukuttan, B., Simmons, E., Landauer, N., Stoddard, J., Hamon, S., Ott, J., Ferguson, B., Klein, M., Stout, J., Neuringer, N. 2008. Rhesus monkeys and humans share common susceptibility genes for age-related macular diseases. *Human Molecular Genetics* 17(17): 2673-2680.

Fride, E. & Weinstock, M. 1988. Prenatal stress increases anxiety related behavior and alters cerebral lateralization of dopamine activity. *Life Sciences* 4(2): 1059-1065.

Gallup, GG 1982. Self-Awareness and the emergence of mind in primates. *American Journal of Primatology* 2: 237-248.

Glaser, D. 2000. Child abuse and neglect and the brain-A review. *The Journal of Child Psychology and Psychiatry* 41(1): 97-116.

Goldsmith, HH, Rothbart, MK. 1991. Contemporary instruments for assessing early temperament by questionnaire and in the laboratory. In Angleitner, A., & Strelau, J. (eds.), *Explorations in Temperament: International perspectives on theory and measurement*. New York: Plenum.

Gunnar, MR & Donzella, B. 2002. Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology* 27: 199-220.

Hammerschmidt, K., Newman, J., Champoux, M., & Suomi, S. 2001. Changes in rhesus macaque 'coo' vocalizations during early development. *Ethology* 106(10): 872-886.

Helfer, ME, Kempe, RS, & Krugman, RD 1997. The battered child. Chicago: University of Chicago Press.

Hinde, RA, & Spencer-Booth, Y. 1967. The behavior of socially living rhesus monkeys in

their first two and a half years. *Animal Behavior* 15: 169-196.

Hussey, J.M., Chang, J. & Kotch, J. B. 2005. Child maltreatment in the United States: Prevalence, risk factors, and adolescent health consequences. *Pediatrics* 118:933-942.

Jadva, V., Hines, M., Golombok, S. 2010. Infants' preferences for toys, colors and shapes: Sex differences and similarities. *Archives of Sexual Behavior* 39(6): 1261-1273.

Kalin, NH, Larson, C., Shelton, SE, & Davidson, RJ 1998. Asymmetric frontal brain activity, cortisol, and behavior associated with fearful temperament in rhesus monkeys. *American Psychological Association* 112: 286-292.

Kalin, NH, & Shelton, SE 2003. Nonhuman primate models to study anxiety, emotion regulation, and psychopathology. *New York Academy of Sciences* 1008:189-200.

Kendall-Tackett, KA & Eckenrode, J. 1996. The effects of neglect on academic achievement and disciplinary problems: A developmental perspective. *Child Abuse & Neglect* 20(3): 161-169.

Lansford, JE, Dodge, KA, Pettit, GS & Bates, JE. 2010. Does physical abuse in early childhood predict substance use in adolescence and early adulthood? *Child Maltreatment* 15(2): 190-194.

Leiter, J. & Johnsen, M. 1994. Child maltreatment and school performance. *American Journal of Education* 102: 154-189.

Lindberg, DG 1971. The rhesus monkey in north India: An ecological and behavioral study. In L.A. Rosenblum (Ed.), *Primate Behavior* 2: 1-106. New York: Academic Press.

Lovejoy, J. & Wallen, K. 1988. Sexually dimorphic behavior in group-housed rhesus monkeys at 1 year of age. *Psychobiology* 16(4): 348-356.

Luthar, S., Cicchetti, D., & Becker, B. 2000. The construct of resilience: A critical evaluation and guidelines for future work. *Child Development* 71(3): 543-562.

Maestripieri, D. 1993. Maternal anxiety in rhesus macaques (Macaca mulatta). *Ethology* 95: 19-31.

Maestripieri, D. 1994. Mother infant relationships in three species of macaques (Macaca mulatta, M. nemestrina, M. arctoides). Development of the mother-infant relationship in the first three months. *Behavior* 131(1): 75-96.

Maestripieri, D. , Wilson, K., & Carroll, K. 1997. Genealogical and demographic influences on infant abuse and neglect in group-living sooty mangabeys. *Developmental Psychobiology* 31(3): 175-180.

Maestripieri, D. & Carroll, KA 1998. Risk factors for infant abuse and neglect in group-living rhesus monkeys. *Psychological Science* 9: 143-145.

Maestripieri, D., & Lindell, S. 2005. Neurobiological characteristics of rhesus macaque abusive mothers and their relation to social and maternal behavior. *Neuroscience & Behavioral* Reviews 29: 51-57.

Maestripieri, D. 2005. Early experience affects the intergenerational transmission of infant

abuse in rhesus monkeys. Proceedings of the *National Academy of Sciences* 27: 9726-9729.

Maestriperi, D., McCormack, K., Higley, JD, Lindell, S., Newman, T., & Sanchez, M. 2006. Early maternal rejection affects the development of monoaminergic systems and abusive parenting in rhesus macaques. *Behavioral Neuroscience* 120: 1017-1024.

Maestripieri, D. 2008. Chapter 11- The role of the brain serotonergic system in the origin and transmission of adaptive and maladaptive variations in maternal behavior in rhesus macaques. *Neurobiology of the Parental Mind* 1: 163-173.

McCartney, K., Owen, MT, Booth, CL, Clarke-Stewart, A., & Vandell, LD 2004. Testing a maternal attachment model of behavior problem in early childhood. *Journal of Child Psychology and Psychiatry* 45(4): 765-778.

McCormack, K. Sanchez, MM, Bardi, M., Maestripieri, D. 2006. Maternal care patterns and behavioral development of rhesus macaque abused infants in the first 6 months of life. *Developmental Psychology* 48: 537-550.

McCormack, K., Newman, TK, Higley, JD, Maestripieri, D., Sanchez MM. 2009. Serotonin transporter gene variation, infant abuse, and responsiveness to stress in rhesus macaque mothers and infants. *Hormones and Behavior* 55: 538-547.

Mears, CE, Harlow, HF. 1975. Play: early and eternal. *National Academy of Science* 72(5): 1878-1882)

Mitchel, G. 1968. Attachment differences in male and female infant monkeys. *Child Development 39(2): 611-620.*

Moriceau, S., & Sullivan, R. 2005. Neurobiology of infant attachment. *Developmental Psychology* 47(3): 230-242.

Morton, N., Browne, K. 1998. Theory and observation of attachment and its relation to child maltreatment: a review. *Child Abuse & neglect* 22(11): 1093-1104.

Mrazek, PJ & Mrazek, DA. 1987. Resilience in child maltreatment victims: A conceptual exploration. *Child Abuse & Neglect* 11: 357-366.

Nachmias, M., Gunnar, M., Mangelsdorf, S., Hornik Parritz, R., Buss, K. 1996. *Child Development* 67(2): 508-522.

O'Connor, T.G., Cameron, J.L. 2006. Translating research findings on early experience to prevention. *American Journal of Preventive Medicine* 6: 175-181.

Parker, KJ, Buckmaster, CL, Schatzberg, AF, & Lyons, DM. 2004. Prospective investigation of stress inoculation in young monkeys. *Archives of General Psychiatry* 51: 993-941.

de Paul, J & Domenech, L. 2000. Childhood history of abuse and child abuse potential in adolescent mothers: A longitudinal study. *Child Abuse & Neglect* 24(5): 701-713.

Ricks, MH 1985. The social transmission of parental behavior: Attachment across generations. In Bretherton, I. & Waters, E. (Eds.), Growing points of attachment theory and research. *Monographs of the society for Research in Child Development* 50: 211-227.

Rogers, J., Shelton, SE, Shelledy, W., Garcia, R., Kalin, NH. 2008. Genetic influences on

behavioral inhibition and anxiety in juvenile rhesus macaques. *Genes, Brain and Behavior* 7: 463-469.

Ryan, RM & Deci, EL. 2000. Self-determination theory and the facilitation of intrinsic motivation, social development, and well-being. *American Psychologist* 55(1): 68-78.

Sanchez, MM, Ladd, CO, & Plotsky, PM 2001. Early adverse experience as a developmental risk factor for later psychopathology:Evidence from rodent and primate models. *Development and Psychopathology* 13: 419-449.

Sanchez, MM, Noble, PM, Lyon, CK, Plotsky, PM, Davis, M., Nemeroff, CB, & Winslow, JT. 2005. Alterations in diurnal cortisol rhythm and acoustic startle response in nonhuman primates with adverse rearing. *Biological Psychiatry* 57: 373-381.

Sanchez, MM 2006. The Impact of Early Adverse Care on HPA Axis Development: Nonhuman Primate Models. *Hormone Behavior* 50: 623–631.

Sanchez, MM, Alagbe, O., Felfer, JC, Zhang, J., Graff, AE, Grand, AP, Maestripieri, D., & Miller, AH. 2007. Activated p38 MAPK is associated with decreased CSF 5-HIAA and increased maternal rejection during infancy in rhesus monkeys. *Molecular Psychiatry* 12: 895-897.

Sanchez, MM & Pollak, SD. 2009. Socioemotional Development following early abuse and neglect: challenges and insights from translational research. In de Haan, M. &

Gunnar, M. (Eds), *Handbook of Developmental Social Neuroscience*. New York: Guilford Publications.

Sanchez, MM, McCormack, K., & Maestripieri, D. 2010. Ethological abuse in rhesus macaques. In *Formative Experiences* (ed.) Worthman, C., Plotsky, P., & Schachter, D. 226-236. New York: Cambridge University Press.

Shonkoff, JP 2005. National Scientific council on the Developing Child 2005. *Excessive stress disrupts the architecture of the developing brain*: Working Paper #3. http://www.developingchild.net

Silk, JB, Alberts, SC, & Altmann, J. 2003. Social bonds of female baboons enhance infant survival. *Science* 302: 1231-1234.

Sugiura, H. 1997. Matching of acoustic features during the vocal exchange of coo calls by Japanese macaques. *Animal Behavior* 55(3): 673-687.

Suomi, S. 2005. Mother-infant attachment, peer relationships, and the development of social networks in rhesus monkeys. *Human Development* 48: 67-79.

Tomaszycki, ML, Davis, JE, Gouzoules, H., Wallen, K. 2001. Sex differences in infant rhesus macaque separation-rejection vocalizations and effects of prenatal androgens. *Hormones and Behavior* 39: 267-276.

Troisi, A. & D'Amato, RD 1983. Is monkey maternal abuse of offspring aggressive behavior? *Aggressive Behavior* 9: 167-173.

Vyt, A. 1989. The second year of life as a developmental turning point: implications for <sensitive> caretaking. *European Journal of Psychology of Education* 4(2): 145-158.

Wallen, K. 1996. Nature needs nurture: the interaction of hormonal and social influences on

the development of behavioral sex differences in rhesus macaques. *Hormones and Behavior* 30: 364-378.

Williamson, DE, Coleman, K., Bacanu, SA, Devlin, BJ, Rogers, J., Ryan, ND 2003.Heritability of fearful-anxious endophenotypes in infant rhesus macaques: a preliminary report 8. *Biological Psychiatry* 53: 284-291.