THE IMPACT OF EARLY MATERNAL MALTREATMENT ON SOCIAL BEHAVIOR, BEHAVIORAL RESPONSES TO STRESS, AND NEUROCHEMICAL SYNTHESIS AND TURNOVER IN JUVENILE RHESUS MACAQUES

by

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(Under the Direction of Irwin Bernstein)

ABSTRACT

Early adverse care has been associated with a variety of negative developmental outcomes, including the development of psychopathologies and impairments in social competence and regulation of emotions. Concurrent alterations in neural threat- and stress-response systems may underlie maltreatment-related socioemotional and cognitive alterations observed in maltreated children and animal models. This study utilized an experimental, cross-fostering design to examine maltreatment-related alterations in social behavior, behavioral responses to a stressor, and synthesis and turnover of specific neuropeptides and neurotransmitters involved in systems associated with the stress response and psychopathologies. Subjects were 36 juvenile rhesus macaques (*Macaca mulatta*) cross-fostered at birth to either maltreating foster mothers (N = 18) or control foster mothers (N = 18). All data were collected when the subjects were 18 months of age. Undisturbed social behavior was measured via focal observations while the subjects were in their social group. Behavioral responses to a stressor were measured during an Approach/Avoidance test during which subjects were presented with a fear-evoking object. CSF samples were collected to measure concentrations of CRF, 5-HIAA,

MHPG, and HVA. In the social group, maltreated subjects exhibited affiliative behavior toward juveniles significantly less than control subjects but were similar in other measures of behavior. During the Approach/Avoidance task, maltreated subjects spent a significantly smaller percentage of time than the control subjects visually inspecting the fear-evoking object potentially indicating that maltreated subjects were less vigilant than control subjects. Maltreated and control subjects were similar on other behavioral responses. CSF concentrations of CRF, MHPG, 5-HIAA, and HVA correlated with behavior observed in the social compound and during the Approach/Avoidance test but did not differ between maltreated and control subjects. Similarly, dimensions of early maternal care (i.e., protectiveness, responsiveness, security, irritability, average rates of abuse and rejection) correlated with behavioral measures with significant group differences, indicating that the early mother-infant relationship likely influences behavioral development later in life. These findings are discussed in terms of the developmental time course over which maltreatment-related alterations in behavior may appear, resilience, temperament, genetic heritability, and potential protective factors against the negative behavioral outcomes associated with experiencing early adverse care.

INDEX WORDS: maternal maltreatment, early adversity, stress, behavior, CRF, DOPAC, 5-HIAA, MHPG, HVA, rhesus

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CHAPTER 1

INTRODUCTION

Early adverse care, particularly childhood maltreatment in the form of abuse and/or neglect, is a serious public health problem in our society (Hussey, Chang, & Kotch, 2005; Sanchez & Pollack, 2009). Childhood maltreatment has been strongly implicated in the development of behavioral problems related to aggression and impulsivity as well as psychiatric disorders, predominantly mood, anxiety, and addictive disorders (Heim & Binder, 2012; Heim & Nemeroff, 2001; Kreppner, O'Connor, & Rutter, 2001; Pesonen & Räikkönen, 2012; Rogosch & Cicchetti, 1994; Sanchez, 2006; Sanchez, Ladd, & Plotsky, 2001). Additionally, childhood maltreatment has been associated with impairments in social competence, regulation of emotions, and cognition (Alessandri, 1991; Hart, Gunnar, & Cicchetti, 1995; Loman & Gunnar, 2010; Pesonen & Räikkönen, 2012; Rogosch, Cicchetti, & Aber, 1995; Sanchez & Pollak, 2009).

Neurobiology Underpinning Maltreatment-Related Socioemotional Alterations

The focus of recent studies, including the present one, has been to better characterize and understand the neurobiology underlying maltreatment-related socioemotional and cognitive alterations by examining maltreatment-related developmental changes in neurochemical synthesis and turnover. A number of studies have examined the role of hypothalamic and extrahypothalamic corticotropin-releasing factor (CRF) systems and of the brain monoaminergic systems in stress reactivity and psychopathology. CRF regulates behavioral, autonomic (sympathetic) and endocrine responses to stressors, including mediation of the HPA axis and sympathetic-adrenomedullary (SAM) activations during the stress response. Some of the

behavioral effects are mediated via regulation of the brain monoaminergic systems (Gunnar & Herrara, 2007; Gunnar & Quevedo, 2007; Gutman & Nemeroff, 2003; Sanchez & Pollak, 2009). The monoaminergic systems of particular interest include the brain serotonergic system, which is involved in emotion regulation and impulse control, the brain noradrenergic system, which is involved in arousal regulation and threat-response, and the brain dopaminergic system, which is involved in reward-related behavior, motivation, behavioral inhibition, and prefrontal cognitive functions (Higley et al., 1992; Higley, Suomi, & Linnoila, 1996; Kaufman, Plotsky, Nemeroff, & Charney, 2000; Maestripieri, McCormack, et al., 2006; Sanchez et al., 2001). Alterations in each of these systems have been implicated in different forms of psychopathology (Gersho, Sudheimer, Tirouvanziam, Williams, & O'Hara, 2013; Heim & Binder, 2012; Heim & Nemeroff, 2001; LaPrairie, Heim, & Nemeroff, 2010; Maestripieri, McCormack et al., 2006; Manji, Drevets, & Charney, 2001; Manuck et al., 1998; McCormack, Newman, Higley, Maestripieri, & Sanchez, 2009; Moghaddam, 2002; Sanchez et al., 2007; Skelton, Weiss, & Bradley, 2010). Investigation of alterations in levels of neurotransmitters and neuropeptides in these systems in response to childhood maltreatment is important in understanding how the experience of early adverse care may sensitize these systems to stress and the development of later psychopathologies.

To assess how adverse early care influences neuropeptide and neurotransmitter synthesis and turnover, researchers often examine cerebrospinal fluid (CSF) concentrations of CRF and monoamine metabolites, which are used as indices of brain CRF activity and monoamine production and turnover (Higley, Suomi, & Linnoila, 1992; Maestripieri, McCormack et al., 2006; Pryce et al., 2005; Shannon et al., 2005). These monoamine metabolites include 5-hydroxyindoleacetic acid (5-HIAA), a serotonin metabolite, 3-methoxy-4-hydroxyphenylgycol

(MHPG), a metabolite of norepinephrine, and homovanillic acid (HVA), a dopamine metabolite (Pryce et al., 2005). Research has demonstrated that alterations in CSF concentrations of CRF and these monoamine metabolites are associated with the occurrence of psychopathology and impairments in both human and animal models (Berman & Coccaro, 1998; Fairbanks, Melega, Jorgensen, Kaplan, & McGuire, 2001; Maestripieri, McCormack, et al., 2006; Manji et al, 2001; Mann, Oquendo, Underwood, & Arango 2009; Melhman et al., 1994; Sanchez & Pollak, 2009).

Nonhuman Primate Model of Early Adverse Care

Infant maltreatment also has been reported to spontaneously occur in nonhuman primates, the closest animal models of childhood maltreatment in humans, in both captive and wild settings (Brent, Koban, & Ramirez, 2002; Johnson, Kamilaris, Calogero, Gold, & Chousos, 1996; Maestripieri, 1999, 1998; Maestripieri & Carroll, 1998a; Maestripieri, Wallen, & Carroll, 1997a,b; Sanchez, 2006; Sanchez & Pollak, 2009; Sanchez, McCormack, & Maestripieri, 2010; Troisi & D'Amato, 1984; Troisi, D'Amato, Fuccillo, & Scucchi, 1982). In studies of early adverse care, rhesus macaques (*Macaca mulatta*) are the most commonly investigated nonhuman primate species (Sanchez, 2006). Similar to the rate of childhood maltreatment in humans, approximately 2-10% of rhesus infants are abused and/or neglected by their mothers (Maestripieri, 1999, 1998; Maestripieri & Carroll, 1998a,b; Maestripieri, Jovanovic, & Gouzoules, 2000). In these cases, physical maternal abuse is operationally defined as violent, infant-directed behavior including the mother crushing, throwing, dragging, and stepping/sitting on the infant (Maestripieri, 1998; Troisi & D'Amato, 1984). This atypical behavior causes significant infant distress, in extreme cases leading to serious physical injury or death, and is distinct from other aggressive behavior which occurs during weaning-related conflicts, such as bites or slaps, and other behavior which is a part of the specie's aggressive repertoire. Maternal

abuse has been observed to occur most frequently during the first 3 months of life, with the highest rates of abuse occurring during the first and second months of life, is repeated with successive offspring, and is more prevalent in some matrilines indicating intergenerational transmission of maternal abuse (Maestripieri, 2005, 1999; Maestripieri, Tomaszycki, & Carroll, 1999; McCormack et al., 2006; Sanchez, 2006). In addition to exhibiting abusive behavior, approximately 70% of abusive mothers exhibit comorbid high rates of infant rejection very early after birth (Maestripieri, 1998; Maestripieri & Carroll, 1998a,b; McCormack, Sanchez, Bardi, & Maestripieri, 2006; Sanchez 2006; Sanchez & Pollak, 2009; Sanchez et al., 2010). Infant rejection is operationally defined as the mother preventing contact or infant access to her nipple by holding the infant at a distance with an arm, passively blocking her chest with an arm, or twisting her torso away from the infant (McCormack et al., 2006). The spontaneous occurrence of infant maltreatment in rhesus monkeys, in addition to its similarity to childhood maltreatment in humans, provides an opportunity to experimentally study the long-term, developmental effects of maternal maltreatment.

The Impact of Maternal Maltreatment on Social Behavior

Studies of abuse and rejection in rhesus monkeys have demonstrated that experiencing maternal maltreatment in infancy leads to socioemotional alterations similar to those observed in maltreated children (Grand, McCormack, Maestripieri, & Sanchez, 2005; McCormack, et al., 2009; McCormack et al., 2006). McCormack and colleagues (2006) found that maltreated infants exhibited a general pattern of delayed social development and increased levels of distress and dependency when compared to controls at 4 to 6 months of age (McCormack et al., 2006). These socioemotional alterations are further associated with alterations in CSF concentrations of monoamine metabolites linked to experiencing high levels of maternal rejection in early infancy

(Maestripieri, Lindell et al., 2006; Maestripieri, McCormack et al., 2006). Juveniles who experienced high rates of early maternal rejection and who had lower levels of 5-HIAA engaged in solitary play more frequently (Maestripieri, McCormack, et al., 2006) and exhibited increased levels of anxiety in the social group (Maestripieri, McCormack, et al., 2006; Sanchez et al., 2007) at 2 years of age. Although not affected by early maternal rejection, lower concentrations of MHPG were associated with increased frequency of solitary play and avoidance behavior at 2 years of age. Since exposure to maternal rejection predicts solitary play but not CSF concentrations of MHPG, Maestripieri and colleagues suggest that socially phobic behavior and fearfulness may be more dependent on genetic inheritance and only weakly affected by early adverse experience (i.e., individuals with low CSF concentrations of MHPG may be more socially phobic and fearful in general, regardless of early experience).

When tested for behavioral reactivity to novel stimuli, abused rhesus juveniles (approximately 24-28 months old) exhibited context-dependent alterations in behavior in comparison to controls which were correlated with low levels of 5-HIAA (Grand et al., 2005). In this study, in the presence of a Human Intruder, abused juveniles displayed more anxious and fearful behavior, consistent with internalizing symptoms reported in studies of neglected, physically abused and sexually abused children, particularly females (Bolger & Patterson, 2001; Keiley, Howe, Dodge, Bates, & Pettit, 2001; Manly, Kim, Rogosch, & Cicchetti, 2001; Young, Abelson, Curtis, & Nesse, 1997). However, abused juveniles were more aggressive/impulsive and less fearful in the presence of novel objects (e.g., shorter latency to investigate fear-evoking objects, more exploration when presented with neutral objects) which also is consistent with externalizing symptoms reported in maltreated children, particularly males (Cicchetti & Rogosch, 2001; Kaplow & Widom, 2007; Manly et al., 2001; Sanchez & Pollak, 2009). These

behavioral alterations may reflect impairments in threat-assessment and/or increased emotional reactivity that manifests as different behavioral responses in different contexts (e.g., an individual may have an underlying state of fear that can manifest as increased freezing in one context, but increased aggression in a different context) (Grand et al., 2005).

Predictions

The present study builds upon and extends this work by examining the developmental effects of early adverse care on behavioral responses to stress, the development of social behavior, and neurotransmitter and neuropeptide levels at 18 months of age in rhesus monkeys. This age represents an important period in rhesus socioemotional development (e.g., increased social interactions with peers which mimic adult social interactions) and neural development (e.g., rapid development of areas vulnerable to early adverse care)(Heim & Binder, 2012; Sanchez et al., 2001; Suomi, 2005). Importantly, the present study used a cross-fostering experimental design with random assignment at birth to either maltreating or control mothers in order to eliminate the potential effects of genetic and prenatal factors on the development of social behavior, behavioral responses to stress, and neuropeptide and neurotransmitter levels. Social behavior was observationally measured while the individuals were undisturbed in their social compounds. Behavioral responses to stress were measured utilizing a standardized laboratory test, the Approach/Avoidance test, which elicits strong, distinct behavioral responses to both neutral and fear-evoking novel objects (Bethea et al., 2004; Machado, Kazama, & Bachevalier, 2009; Meunier et al., 1999; Williamson et al., 2003). Finally, CSF samples were collected to examine whether maternal maltreatment has long-term effects on neurotransmitter and neuropeptide synthesis and turnover in the serotonergic, dopaminergic, noradrenergic, and CRF systems. The aim of the present study was twofold:

1. To characterize the long-term effects of early maltreatment on the development of social behavior and behavioral responses to stress and to examine whether these effects differ based upon the sex of the individual as suggested by the human literature. Based on the previous literature, it was predicted that maltreated juveniles would exhibit behavioral alterations in the social group with females exhibiting more sociophobic/avoidant behavior, males more aggressive behavior, and both sexes exhibiting more anxiety-related behavior. It also was predicted that maltreated juveniles would exhibit more aggressive and fearful behavior than controls during the Approach/Avoidance test, particularly maltreated males.
2. To characterize the effects of experiencing early maltreatment on long-term alterations in neural systems which are influenced by stress- and threat-response systems by examining neurotransmitter synthesis and turnover of these systems, including whether there are sex differences in neuropeptide and neurotransmitter levels. It was predicted that juveniles that experience early adverse care would have higher CSF concentrations of CRF and lower CSF concentrations of 5-HIAA, HVA, and MHPG. It also was predicted that the levels of CRF and these monoamine metabolites would correlate with behavior observed in the social compound and during the Approach/Avoidance test.

CHAPTER 2

METHOD

Subjects and Housing

Subjects were 36 juvenile rhesus macaques, 10 (4 male, 6 female) born between April and May 2009, 13 (9 male, 4 female) born between April and June 2010, and 12 (6 male, 6 female) born between April and June 2011. They were socially housed (see Table 1 for group compositions) in compounds consisting of an outdoor enclosure with an adjacent indoor run at the Yerkes National Primate Research Center (YNPRC) Field Station in Lawrenceville, Ga. Each social group had a stable matrilineal structure and dominance hierarchy. Water was available *ad libitum* and monkey chow was provided twice per day. Additionally, fresh fruit and enrichment food items were provided once per day. All testing, behavioral observations, and sample collections were done when the subjects were 18 months old. All procedures described below were approved by the Emory Institutional Animal Care and Use Committee and were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Table 1: Social group composition for each cohort.

Foster Group at Birth	**Number of Subjects in Study**	**Approximate Foster Group Composition for first year of life***
2009 Cohort		
1	1	6-8 adult males; ~100 females with their sub-adult and juvenile offspring
2	4	2 adult males; 60-70 adult females with their sub-adult and juvenile offspring
3	4	3-5 adult males; unknown number of adult females or total group size**

4	1	2-3 adult males; ~40 adult females with their sub-adult and juvenile offspring
2010 Cohort		
1	4	6-8 adult males; ~100 adult females with their sub-adult and juvenile offspring
2	4	2 adult males; 60-70 adult females with their sub-adult and juvenile offspring
3	5	3-5 adult males; unknown number of adult females or total group size**
2011 Cohort		
1	6	6 adult males; 84 adult females with their sub-adult and juvenile offspring
2	2	2 adult males; 38 adult females with their sub-adult and juvenile offspring
3	4	5 adult males; 41 adult females with their sub-adult and juvenile offspring

*Adult males were removed from the social groups due to other institutional protocols in August & September 2012.
**The number of females and total group size was missing from the 2009 and 2010 colony records for group 3.

Prior to this study and within the first two days of life, each subject was randomly assigned to be reared by a multiparous foster mother. These females had been identified as either maltreating or non-maltreating (controls) based on their observed and documented maternal behavior in prior birth seasons. This was further confirmed post cross-fostering via focal observations of the infant-mother pair for 30 minutes, 5 times per week during the first month of life, and then twice a week during the second month and once a week from the third month through the sixth month. Maternal maltreatment in this model consisted of two behavioral responses: physical abuse and early infant rejection. Maternal abuse was operationally defined as at least three occurrences of the following violent, infant-directed actions: dragging, crushing, rough grooming, throwing, stepping or sitting on, or roughly carrying the infant (Maestripieri,

2005; McCormack et al., 2006; see Table 2 for definitions of these actions). This behavior causes significant infant distress, however, extremely abusive females (e.g., abuse leads to serious injury or death of the infant) were excluded from the study due to ethical concerns. Maternal rejection was operationally defined as the following actions: preventing 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 from the infant (McCormack et al., 2006). Additional dimensions of maternal care measured for both control and maltreating mothers during the first 3 months of the infant's life included rates of maternal restraint (mother prevents the infant from breaking contact by holding its leg or tail; McCormack et al., 2006), as well as measures of maternal responsiveness, protectiveness, security, and irritability using a Laboratory Protectiveness Scale modified for use with nonhuman primates (see Appendix A). Observers completed the Laboratory Protectiveness Scale for each mother-infant pair immediately following each focal observation conducted during the first 3 months of life. Ten subjects (7 male, 3 female) were in the control/maltreatment cross-fostered group (i.e. biological infant of a control mother cross-fostered to a maltreating mother), 7 subjects (2 male, 5 female) were in the maltreatment/control group (i.e. biological infant of a maltreating mother cross-fostered to a control mother), 12 subjects (6 male, 6 female) were in the control/control group (i.e. biological infant of a control mother cross-fostered to a control mother), and 8 subjects (5 male, 3 female) were in the maltreatment/maltreatment group (i.e. biological infant of a maltreating mother cross-fostered to a maltreating mother).

Table 2: Maternal abuse ethogram.

Behavior	**Operational Definition**
Drag	Drags infant by its tail while walking or running
Crush	Pushes infant against the ground with hands

Throw	Throws the infant ahead while walking or running
Step or sit on	Steps or sits on infant
Rough groom	Holds infant into the ground and pulls out infant's hair with force
Rough carry	Carries infant with one arm away from her body, infant unable to cling

Procedure

Cross-Fostering

Cross-fostering was used in order to rule out the potential confound of genetic and prenatal factors on developmental outcomes. Previous research has demonstrated that cross-fostering is feasible with rhesus macaques and that cross-fostering can be accomplished using the methods described below without affecting the foster mothers' parenting behavior (Maestripieri, Megna, & Jovanovic, 2000; Maestripieri, 2005; Maestripieri, Lindell, & Higley, 2007). At birth, infants were randomly assigned to one of the groups described above.

Rhesus macaques are seasonal breeders, with breeding occurring in the fall and births occurring in the spring. At YNPRC, the birth season is typically March through May, increasing the likelihood of matching infants for cross-fostering. Prior to the breeding season, a list of potential foster mothers was identified using YNPRC and laboratory records. Multiparous females with and without a history of maltreating their infants were selected. Control females were matched to maltreated females based on their social group and rank. Prior to the birth seasons, ultrasounds were performed to aid in subject assignment by verifying pregnancies, estimating birth dates, and checking on fetal health. Cross-fostering was done within 48 hours of birth with multiparous females living in different social groups and mother-infant separations

were never more than 5 minutes during the cross-fostering procedure (per Maestripieri et al., 2000; Maestripieri, 2005). This, as well as having strong involvement of YNPRC veterinary and colony management staff, has been shown to be the key factors in successfully cross-fostering rhesus macaques at YNPRC, including the cross-fosters which were done for this study (Guzman et al., 2012; Howell et al., 2011, 2012; Sanchez et al., 2011, 2012).

Behavioral Data Collection in the Social Group

Four, 30 minute focal observations were carried out for each subject from an observation tower or the ground outside the compound. Observations were conducted between 8 and 11am, when the animals were most active. No more than one observation per subject was conducted in a single day. In order to obtain undisturbed behavior, observations for each subject were not conducted in the same day that CSF samples were collected or that the subject participated in the Approach/Avoidance test. The entire social group was locked in the outdoor enclosure during the observation sessions. Two experienced observers collected behavioral data utilizing modifications of a well-established, comprehensive ethogram (Altmann, 1962; Hinde & Spencer-Booth, 1967; see Appendix B), binoculars, and a laptop computer with in-house WinObs software (Graves & Wallen, 2006). Prior to data collection, the observers were trained until they reached intra- and inter-rater reliability of greater than 90% agreement and Cohen's Kappa exceeded 0.8.

Training and Capture

Prior to this study, all subjects had been trained and habituated to the procedures for capture, CSF sampling, and Approach/Avoidance testing. The animals were trained to move on command from the outdoor enclosure to an indoor capture unit and from the capture unit to a transfer box. For this study they then either moved from the transfer box to a squeeze cage where

they were anesthetized for CSF collection or remained in the transfer box to be transported to the Approach/Avoidance testing room. Additionally, prior to this study, all subjects had experienced the CSF procedure and a Human Intruder behavioral test similar to that for Approach/Avoidance at 6 and 12 months of age. Even with training, the capture procedure has been demonstrated to activate the stress response in juvenile rhesus macaques (Sanchez et al., 2010).

Behavioral Responses to Stress Data Collection

Behavioral responses to stress were measured, on a day prior to the day CSF samples were collected, using an Approach/Avoidance test. This test was designed to measure behavioral responses to a nonsocial novel stimulus (fear-evoking and neutral objects), particularly the conflict between exploratory behavior (i.e., approaching a food reward in close proximity to the novel object) and behavioral inhibition (i.e., avoidance of the novel, potentially threatening object) (Bethea et al., 2004; Machado et al., 2009; Meunier et al., 1999; Williamson et al., 2003). This study focused on the subjects' behavioral responses to one particular fear-evoking object, a mechanical stuffed pig which moved and "oinked". The mechanical toy pig was chosen because it possesses a number of characteristics that had been demonstrated to evoke fearful and defensive behavior in previous studies of rhesus monkeys (Bethea et al., 2004; Grand et al., in preparation; Williamson et al., 2003).

Each subject experienced one testing session following the capture procedures outlined above. The subjects' behavioral reactions to the novel objects presented were video-taped via a digital camera set up approximately 2m from the front of the testing cage. The testing apparatus consisted of a clear, plexi-glass box (30 x 30 x 30cm) attached to the outside of the testing cage on a side which consisted of vertical bars. These bars allowed the subjects to reach both a jelly bean food reward (placed in front of the object) and the objects in the box, but the bar spacing

was not large enough to allow the objects to be pulled into the cage. A metal screen was slid between the testing cage and the box to prevent the subjects from being able to see the researchers setting up the object and jelly bean before each trial began.

Immediately following transfer from the transfer box to the testing cage, the session began with a 5 minute habituation period. This served to habituate the subject to the testing cage, tray, and room and to show them the jelly bean before object presentations. Each subject was then presented with the following objects for 5 minutes each in the following order with the trial of interest occurring last: plastic cup (neutral), Elmo toy with large eyes (fear-evoking), coiled hose (neutral), coiled rubber snake (fear-evoking), plastic bucket (neutral), and mechanical pig which moved and "oinked" (fear-evoking). Whether an object was categorized as neutral or fear-evoking was based on previous studies of rhesus monkeys (Bethea et al., 2004; Grand et al., in preparation; Williamson et al., 2003). Each session lasted approximately 35 minutes. Immediately following the end of the session, each subject was returned to their social group. For each subject, the videotape of the pig trial was scored for measures of fear-related/defensive behavior, anxiety-related behavior (i.e., displacement/self-directed behavior), aggressive behavior, submissive behavior, stereotypies, latency to eat the jelly bean, and exploratory/object-directed behavior (see Table 3 for the ethogram) using Observer XT (version 10.5, Noldus Information Technology, The Netherlands).

Table 3: Behavioral ethogram for Approach/Avoidance test.

Behavior	**Operational Definition**
Fearful/Defensive	
Freeze	Subject has tense posture with ventrum pressed down to or towards the bottom of the cage. Arms and legs are pressed into the body. Remains for 3 seconds. Or subject is tense, silent and motionless except for slow head movements. (Duration).
Withdrawal	Subject makes a quick, jerky motion to the back of the cage,

	away from the object. New bout after 3 second pause (Frequency).
Grimace	Subject pulls back lips to expose clenched teeth. New bout after 3 second pause (Frequency).
Scream/Screech	High pitched vocalization. New bout after 3 second pause (Frequency).
Displacement/Self-Directed	
Self Groom	Subject picks through own fur. (Duration).
Yawn	Mouth open wide with teeth exposed. New bout after 3 second pause (Frequency).
Scratch	Subject draws its fingernails or toenails across its fur. New bout after 3 second pause. (Frequency).
Body Shake	Shaking of head and shoulder region of the body, like dog shaking off. New bout after 3 second pause (Frequency).
Aggressive	
Cage Agitation	Subject slaps, bites, or shakes cage. (Duration).
Threat	Includes **open mouth** (staring with mouth and eyes wide open), **bob** (head moves up and down or side to side with direct eye contact, **lunge** (subject makes quick forward movement towards in slightly crouched position with direct eye contact, and **raised eyebrow** (direct eye contact with eyes wide open, without open mouth). New bout after 3 second pause (Frequency).
Bark/Threat	Vocalizations made by forcing air from the abdomen through the vocal chords, producing a short, rasping, low frequency sound with threat (see above). New bout after 3 second pause (Frequency).
Submissive	
Lipsmack	Quick movement of jaw pressing lips together. (Duration).
Present	Subject presents rear to object/ intruder. Subject is slightly crouched with knees in locked position. New bout after 3 second pause (Frequency).
Exploration/Object Manipulation	
Object inspect	Subject is watching (visual fixation) object for at least 3 seconds. (Duration and latency to).
Object manipulate	Subject manipulates object by touching object or grabbing the

	object. Not slap (not vigorous and can be maintained for long period of time). (Duration and latency to).
Sniff object	Subject smells object. (Duration and latency to).
Bite object	Subject puts teeth on or around object, applying pressure to object. (Duration and latency to).
Slap Object	Subject makes brief and vigorous contact with object with hand. New bout after 3 second pause (Frequency).
Eat Jelly Bean	Subject puts jelly bean in mouth and chews. (Latency to).
Other	
Stereotypies	Repetitive, motor pattern (e.g. circling, rocking, pacing, head turning) that occurs 3 or more consecutive times. New bout if no stereotypy for 3 seconds. Can only count if at least 3 seconds and occurs at least 3 or more times. (Duration).

CSF Sample Collection and Analysis

CSF samples were obtained from the 2009 and 2010 cohorts, from a total of 24 subjects. All subjects had prior CSF samples collected at 6 and 12 months of age as part of a previous study. All samples were collected at sunrise within 15 minutes of initial disturbance (i.e., researchers' arrival outside the subject's compound). Subjects were captured utilizing the procedures described above and anesthetized with telazol (5-6mg/kg body weight, i.m.) immediately following transfer from a transfer box to a squeeze cage. One 0.5-1ml sample, collected as 2 aliquots of 500µl each, were collected via gravity from the cistern magna with a sterile, 25-gauge needle following published protocols (Maestripieri, McCormack, et al., 2006; Sanchez et al., 2007; Winslow et al., 2003). CSF samples were immediately stored in dry ice and then kept at -80°C until assayed. To determine CRF concentration, samples were analyzed with a peptide enzyme immunoassay (EIA) from Bachem (Peninsula Labs, San Carlos, CA) using an anti-human/rat CRF antibody. All inter- and intra-assay variabilities were less than 10%. Additionally, to determine concentrations of 5-HIAA, HVA, and MHPG, the samples were

analyzed using high performance liquid chromatography with electrochemical detection (HPLC-EC).

Data Analysis

Data were checked for normality and homogeneity of variance prior to statistical analyses. Non-normally distributed data were transformed using a $\log_{10}(x+1)$ transformation. Two-way ANOVA analyses were conducted with gender and foster group assignment as fixed factors. Cohort, rank, and biological mother were included as covariates when one-way ANOVA analyses indicated a significant effect of these variables on behavioral and neurochemical measurements. When transformed measures violated assumptions of normality and/or homogeneity of variance, both two-way ANOVA analyses with a Keppel correction ($\alpha/2$; Keppel & Wickens, 2004) and nonparametric Mann Whitney U analyses comparing control to maltreated subjects were conducted. Chi-square analyses were conducted on categorical data. Simple linear regression analyses were conducted with a Bonferroni correction to examine whether dimensions of early maternal care (during the first 3 months of life) predict behavioral and neurochemical outcomes. Alpha levels were set at 0.05 and all analyses were conducted using SPSS, version 17.0.

Behavioral Data

All behavioral data were collected as durations, frequencies, or latencies. For social behavior, only behavior exhibited by the test subjects toward their peers (juveniles and infants) and their mother were analyzed. Behavior exhibited toward peers was collapsed across juveniles and infants. When significant differences were found, behavior exhibited toward juveniles and behavior exhibited toward infants were separately analyzed. Thirty six subjects were included in the social behavior analyses with the exception of affiliative behavior exhibited toward mother

(N = 34). Two subjects (a control male and a maltreated male) were dropped from the analyses of social behavior toward mother since both subjects' mothers had been removed from the social group and euthanized due to chronic illness prior to the behavioral observations. Thirty five subjects were included in the analyses of behavioral responses to stress during the Approach/Avoidance test. One subject (a control female) did not complete the Approach/Avoidance test due to chronic illness. For both social behavior and behavioral responses to stressors, behavioral measures were analyzed individually and as composite scores (see Tables 3 & 4). Low occurrence behavioral categories or responses (i.e., not exhibited by 50-70% of the subjects) were converted from durations or frequencies to categorical data (yes or no) based on whether each subject exhibited the behavior. Behavior exhibited by 20% or fewer of the subjects were included in composite scores but were not individually analyzed.

Table 4: Composite scores for measures of behavior in the social group.

Composite	**Actions/Behavioral Responses**
Agonistic behavior toward juveniles and/or infants	Sum of bite, chase, harass, threat, noncontact aggression, and contact aggression (Frequency).
Affiliative behavior toward juveniles and/or infants	Frequency: sum of groom solicit, genital inspect, mount, play solicit and touch Duration: sum of contact and groom
Affiliative behavior toward mom	Sum of contact, groom, and proximity (Duration).
Submissive behavior	Sum of present, lipsmack, and avoidance (Frequency).
Anxiety-related behavior	Sum of yawn, body shake, self-groom, and scratch (Frequency).

CSF Data

Twenty two subjects (2009 and 2010 cohorts) were included in the analyses of monoamine metabolites and CRF. CSF samples were not collected from two subjects (a control

female and a control male) due to chronic illness. Assay data were first examined for outliers of greater than 2 standard deviations from the mean. Analyses were then conducted both with and without outliers removed. Two-tailed, parametric Pearson's r correlations (when both variables were normally distributed) or Spearman's rank correlations (when both variables were not normally distributed) were conducted to determine whether the monoamine metabolites and CRF were related to behavioral measures.

Dimensions of Early Maternal Care

Regression analyses for dimensions of early maternal care were limited to only those behavioral and CSF measures that had significant group, sex, or group by sex differences.

CHAPTER 3

RESULTS

Behavior in the Social Group

Control subjects (M = .61, SEM = .06) exhibited affiliative behavior towards juveniles more frequently than maltreated subjects (M = .30, SEM = .06), indicating a significant main effect of group (maltreated, control) on frequency of affiliative behavior toward juveniles ($F(1,32) = 14.77$, $p = .001$, partial $\eta^2 = .32$)(Figure 1). Control subjects (M = .20, SEM = .03) also play solicited juveniles more frequently than maltreated subjects (M = .05, SEM = .04), indicating a significant main effect of group on frequency of play solicits of juveniles ($F(1,32) = 8.84$, $p = .006$, partial $\eta^2 = .22$)(Figure 2). There were no significant interaction effects or main effects of sex on frequency of affiliative behavior toward juveniles or play solicits of juveniles.

Chi-square analyses indicated that the control group avoided infants significantly more than the maltreated group ($\chi^2 = 14.25$, $p = .001$). Post-hoc analyses revealed that this difference was driven by maltreated males; no maltreated males exhibited this behavior in comparison to 75% of control males ($\chi^2 = 12.857$, $p = .003$) whereas no differences were found between maltreated females and control females ($\chi^2 = 1.778$, $p = .307$)(Figure 3). Additional Chi-square analyses revealed no significant group or group by sex differences for grooming juveniles and infants, mounting juveniles, play solicits of infants, tantrums, ventral and other contact with mother, and agonistic behavior exhibited toward juveniles, specifically harass, noncontact aggression, total aggression, and agonistic behavior ($p > .05$).

The following behavioral measures were exhibited by 20% or less of the subjects and were not included in individual analyses: attack, bite, chase, contact aggression, dorsal contact with mom, coo, cradle, genital inspect, grimace, hit, kidnap, lipsmack, present, and yawn. Two-way ANOVA analyses and Mann Whitney U analyses revealed no significant group, sex, or group by sex effects or differences for the remaining behavioral measures ($p > .05$).

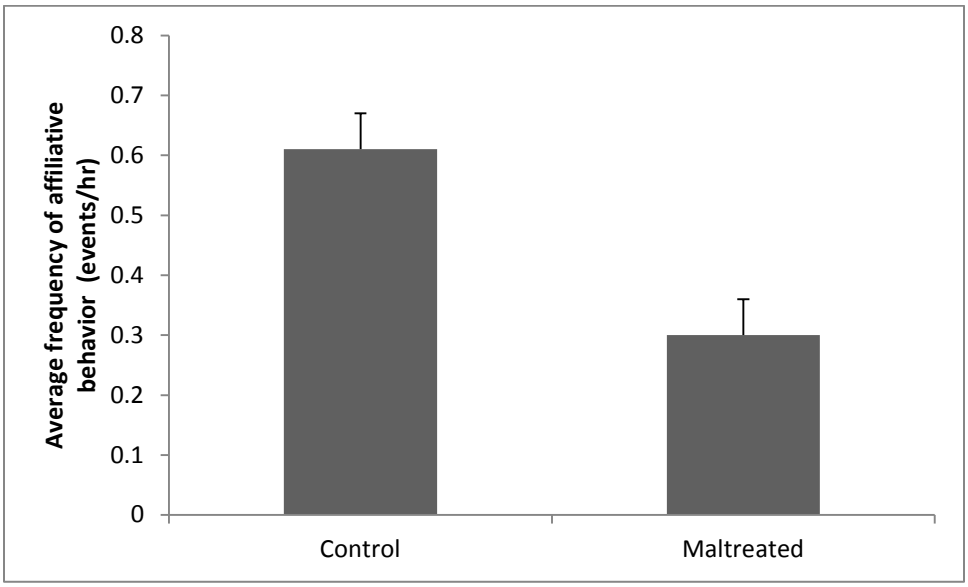

Figure 1: Average frequency of total affiliative behavior displayed by subjects toward other juveniles. Control subjects (M = .61, SEM = .06) exhibited affiliative behavior towards juveniles more frequently than maltreated subjects (M = .30, SEM = .06)($F(1,32) = 14.77$, $p = .001$, partial $\eta^2 = .32$).

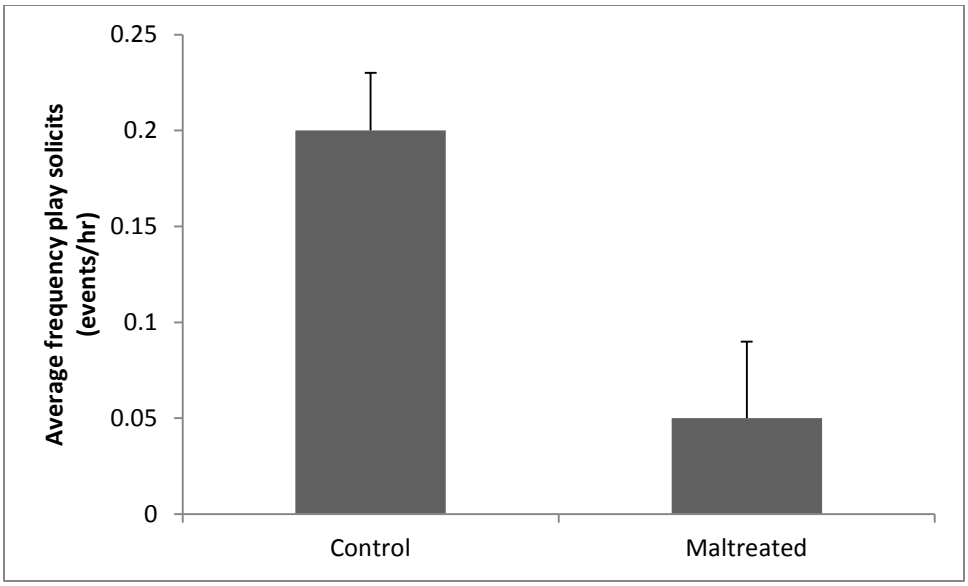

Figure 2: Average frequency of play solicits of other juveniles. Control subjects (M = .20, SEM = .03) play solicited juveniles more frequently than maltreated subjects (M = .05, SEM = .04)($F(1,32) = 8.84$, $p = .006$, partial $\eta^2 = .22$)

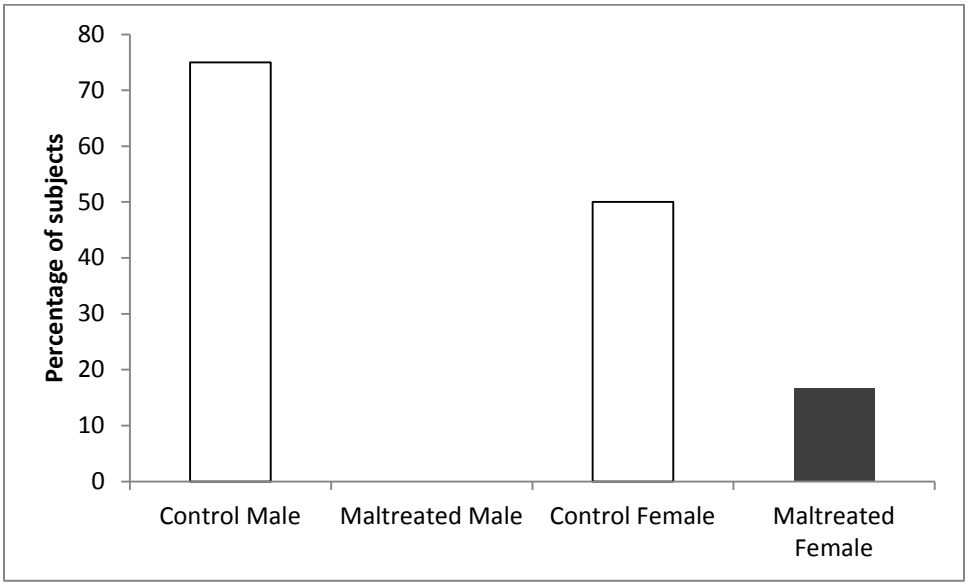

Figure 3: Percentage of subjects that avoided infants in the social group. No maltreated males avoided infants in comparison to 75% of control males ($\chi^2 = 12.857$, $p = .003$) whereas no significant differences were found between maltreated females and control females ($\chi^2 = 1.778$, $p = .307$).

Behavior Responses during the Approach/Avoidance Test

Control subjects (M = 4.34, SEM = 1.24) spent a greater percentage of time inspecting the pig than maltreated subjects (M = 1.2, SEM = .61), indicating a significant effect of group assignment on percentage of time spent inspecting the pig between control subjects and maltreated subjects (U = 82, z = -2.49, p = .013)(Figure 4). No significant group, sex, or group by sex effects or differences were found for percentage of time spent sniffing the pig, exploration/objection manipulation, aggressive/dominant behavior, or latencies to inspect the pig, sniff the pig, and eat the jelly bean ($p > .05$).

Chi-square analyses revealed no significant group or group by sex differences for freeze, manipulate pig, cage agitation, bite pig, motor stereotypies, bark threat, slap pig, threat, withdrawal, scream, or composite scores of submissive behavior, displacement/self-directed behavior, and fearful/defensive behavior ($p > .05$). Lipsmack, grimace, present, yawn, body shake, and self-groom were not included in individual analyses since greater than 80% of the subjects did not exhibit these behavioral responses during testing.

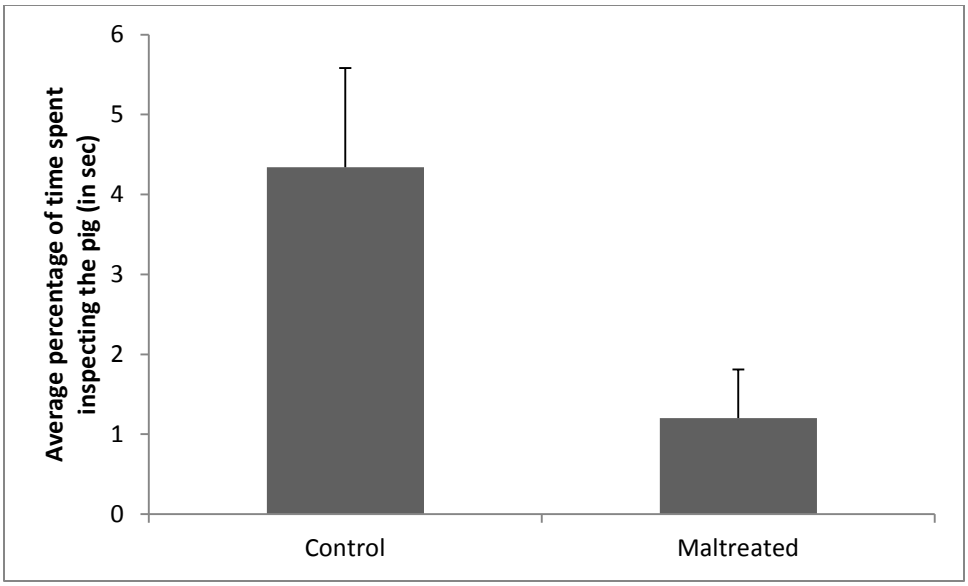

Figure 4: Average percentage of time subjects spent inspecting the pig during the Approach/Avoidance test. Control subjects (M = 4.34, SEM = 1.24) spent a greater percentage of time inspecting the pig than maltreated subjects (M = 1.2, SEM = .61)(U = 82, z = -2.49, p = .013).

CSF Levels of CRF, 5-HIAA, HVA, and MHPG

MHPG and CRF each had one outlier greater than 2 standard deviations from the mean. Two-way ANOVA analyses indicated no significant group, sex, or group by sex effects for CSF concentrations of CRF, 5-HIAA, HVA, or MHPG ($p > .05$).

Correlation Analyses

CSF Measures and Behavior in the Social Group

CRF concentrations and anxiety-related behavior were positively correlated ($r = .543$, $p < .05$). Similarly, levels of MHPG with outliers removed was positively correlated with frequency of scratching ($r = .427$, $p < .01$). Duration of contact with infants was negatively correlated with levels and of 5-HIAA($r_s = -0.432$, $p < .05$) and HVA ($r_s = -.472$, $p < .05$). Similarly, frequency of affiliative behavior toward infants was negatively correlated with levels of 5-HIAA ($r_s = -.510$, $p < .05$) and HVA ($r_s = -.534$, $p < .01$). Additionally concentrations of 5-HIAA were negatively

correlated with frequency of noncontact aggression (r_s = -.488, $p < .05$) and total agonistic behavior (r_s = -.450, $p < .05$) directed toward infants. There were no other significant correlations between any of the CSF measures (both with and without outliers removed) and the remaining behavioral measures ($p > .05$).

CSF Measures and Behavioral Responses during the Approach/Avoidance Test

Spearman's rank analyses revealed significant positive relationships between CRF and displacement/self-directed behavior (r_s = .428, $p = .05$), CRF and percentage of time spent inspecting the pig (r_s = .428, $p = .05$), MHPG and fearful/anxiety-related behavior (r_s = .484, $p = .02$), and MHPG and percentage of time spent manipulating the pig (r_s = .463, $p = .03$). There were no other significant correlations between any of the CSF measures (both with and without outliers removed) and the remaining behavioral responses ($p > .05$).

Dimensions of Early Maternal Behavior as Predictors of Social Behavior

No dimension of early maternal behavior significantly differed between maltreating and control mothers. However, specific dimensions of early maternal behavior did correlate with behavioral measures which significantly differed between maltreated and control subjects. Frequency of affiliative behavior toward juveniles was significantly correlated with average maternal responsiveness ($\beta = .569$, $t(36) = 4.04$, $p = .000$) and protectiveness ($\beta = .488$, $t(36) = 3.26$, $p = .003$) over the first 3 months of life, indicating a positive relationship between affilative behavior toward juveniles and these dimensions of maternal care. Average maternal responsiveness accounted for 30.4% of the variance in affiliative behavior ($F(1,36) = 16.32$, $p = .000$) while average maternal protectiveness accounted for 21.6% of the variance in affiliative behavior ($F(1,36) = 10.62$, $p = .003$). Similarly, frequency of play solicits of juveniles was significantly correlated with maternal responsiveness ($\beta = .524$, $t(36) = 3.59$, $p = .001$) and

average maternal security (e.g., degree to which mother serves as a secure base)(β = .440, t(36) = 2.86, p = .007), indicating a positive relationship between play solicits and these dimensions of maternal care. Average maternal responsiveness explained 25.3% of the variance in play solicits (F(1,36) = 12.86, p = .001) while average maternal security explained 17% of the variance in play solicits (F(1,36) = 8.15, p = .007). Additionally, average maternal irritability was significantly correlated with frequency of affiliative behavior (β = -.566, t(36) = -4.00, p = .000) and play solicits (β = -.478, t(36) = -3.18, p = .003), indicating negative relationships between maternal irritability and play solicits and affiliative behavior and play solicits. Average maternal irritability explained 30 % of the variance in frequency of affiliative behavior (F(1,36) = 16.01, p = .000) and 20.6% of the variance in frequency of play solicits (F(1,36) = 10.01, p = .003).

Spearman's rank analyses revealed that maternal abuse was negatively correlated with frequency of affiliative behavior toward juveniles (r_s = -.528, p = .001) and frequency of play solicits of juveniles (r_s = -.455, p = .005). Additionally, avoidance of infants was positively correlated with maternal responsiveness (r_s = .562, p = .000), maternal security, (r_s = .453, p = .005), and maternal protectiveness (r_s = .588, p = .000) but was negatively correlated with maternal irritability (r_s = -.464, p = .004) and average rate of maternal abuse (r_s = -.492, p = .002).

Dimensions of Early Maternal Behavior as Predictors of Behavioral Responses in the Approach/Avoidance Test

Spearman's rank analyses also indicated that the percentage of time spent visually inspecting the pig during the Approach/Avoidance task was positively correlated with only one dimensions of early maternal care, average maternal responsiveness (r_s = .470, p = .004). Time

spent inspecting the pig also was negatively correlated with average maternal irritability (r_s = -.461, p = .005) and average rates of maternal rejection (r_s = -.457, p = .006).

Dimensions of Early Maternal Behavior as Predictors of CSF Measures

No dimension of early maternal care predicted any of the CSF measures (p > .05). Pearson's r correlations also indicated no correlation between dimensions of maternal care and the CSF measures (p > .05).

CHAPTER 4

DISCUSSION

Evidence from this study indicates that the early mother-infant relationship likely influences the development of social behavior and behavioral responses to stressors in rhesus macaques as the following dimensions of early maternal behavior correlated with these measures: maternal responsiveness, maternal protectiveness, maternal security, maternal irritability, and average rates of abuse and rejection. There also is evidence that rhesus monkeys who experience early maternal maltreatment exhibit long-term behavioral alterations in specific aspects of social behavior and behavioral responses to a stressor, namely: frequency of affiliative behavior and play solicits directed toward juveniles and avoidance of infants in the social group and the percentage of time spent inspecting the pig during the Approach/Avoidance test. However, the results did not indicate that experiencing early maltreatment leads to long-term alterations in the synthesis and turnover of CRF, 5-HIAA, HVA, and MHPG.

Social Behavior

There was a significant difference between control and maltreated subjects in frequency of affiliative behavior directed toward peers. Control subjects both play solicited other juveniles more often as well as exhibited overall affiliative behavior more frequently toward other juveniles in comparison to maltreated subjects. This difference in overall affiliative behavior is consistent with studies of behavioral outcomes earlier in rhesus development. McCormack and colleagues demonstrated maltreatment-related behavioral alterations indicative of a general delay in social development during the first 6 months of life (2006, 2003). However, abused subjects in

McCormack and colleagues' (2006) study spent more time in solitary play than controls, which was not the case in this study. The finding that maltreated subjects exhibited affiliative behavior and play solicited their peers less often in this study alone may not be indicative of sociophobic behavior in maltreated subjects but does suggest that at 18 months of age there remain differences in affiliative behavior as a result of experiencing early maltreatment.

Research examining maltreatment-related alterations in social behavior later in rhesus development indicates a further decline of alternations in affiliative behavior. Grand and colleagues (2005) did not find any differences in affiliative behavior between abused and control subjects at 24-28 months of age in their study. Although McCormack and colleagues (2006) and Grand and colleagues (2005) did not use a cross-fostering experimental design, they did match their control and maltreating subjects on a number of characteristics (e.g., infant sex and age, maternal rank and social group when possible) and their methods for measuring maternal maltreatment and infant behavior were similar to the present study, indicating that the differences between studies were unlikely due to methodological differences. This suggests that maltreated rhesus infants may begin to recover from maltreatment-related delays in social development observed earlier in life by 18 months of age and that these alterations may completely disappear by 24-28 months of age.

Similarly, not all maltreated children exhibit the negative behavioral outcomes associated with experiencing early adverse care, indicating that some children are more resilient than others (for a review see Afifi & MacMillan, 2011). In their review, Afifi and MacMillan identify protective factors which have been associated with resilience in children. One protective factor strongly associated with resilience in children and most relevant to the nonhuman primate studies is that of supportive relationships. Evidence from the human literature indicates that positive peer

relationships may moderate the deficits in social development associated with early maltreatment (Bolger, Patterson, & Kupersmidt, 1998). Bolger and colleagues found that, for some chronically maltreated children and physically abused children, having a high quality friendship, or more reciprocated playmates, were associated with greater increases of self-esteem over time. As in human children, positive peer relationships may help maltreated juvenile macaques recover from any maltreatment-related behavioral alterations or delays in social development observed earlier in life. Future research should focus on measuring resilience in nonhuman primates, including potential rehabilitative or protective factors such as positive peer relationships. Both quantitative and qualitative aspects of social relationships between maltreated subjects and social group members during development should be measured to examine whether positive social relationships serve to ameliorate negative behavioral outcomes associated with experiencing early adverse care in nonhuman primates.

Contrary to predictions, maltreated subjects did not exhibit more aggressive behavior in the social group in comparison to controls. Similarly, McCormack and colleagues (2006) found no differences in aggressive behavior between control and abused subjects at 6 months of age. But, Grand and colleagues (2005) found that abused subjects displayed more aggressive behavior than control subjects, including noncontact aggression and contact aggression, and were more likely than control subjects to respond to aggression with aggression at 24-28 months of age (Grand et al., 2005). This may indicate a developmental progression in aggressive behavior in which maltreatment-related alterations of this behavior do not appear until later in rhesus development, closer to puberty.

Overall, the predictions that maltreated females would exhibit more sociophobic/avoidant behavior, maltreated males more aggressive behavior, and maltreated juveniles more anxiety-

related behavior were not supported by the results. There were no group, sex, or group by sex differences in frequency of avoidance behavior with the exception that abused males never avoided infants. Additionally, there were no differences in durations or proportion of social or solitary play or durations or frequencies of any measures of anxiety-related behavior and aggressive behavior between both control and maltreated subjects and male and female subjects.

Behavioral Responses during the Approach/Avoidance Test

There was a significant difference between maltreated and control subjects in percentage of time spent visually inspecting the pig during the Approach/Avoidance task. Previously, both control and abused juveniles spent more time visually inspecting fear-evoking objects (Grand et al., in preparation). In this study, control subjects spent a greater percentage of time inspecting the pig in comparison to maltreated subjects, indicating greater vigilance on the part of control subjects. This could be indicative of less fearfulness/behavioral inhibition and impairments in threat assessment in maltreated subjects, however, maltreated and control subjects did not differ in other behavioral responses which serve as better indicators of fearfulness/behavioral inhibition (e.g., freezing or withdrawal) (Kalin & Shelton, 1989).

There is some indication that abused juveniles (at 2 years of age) were more aggressive and impulsive when presented with fear-evoking objects during an Approach/Avoidance test (Grand et al., in preparation). However, the prediction that maltreated subjects would exhibit more aggressive and fearful behavior during the Approach/Avoidance test was not supported by the results in the present study. There were no group, sex, or group by sex differences in any of the behavioral responses to a fear-evoking object in this study, with the exception of percentage of time spent inspecting the pig. These findings are not consistent with the human literature which indicates that abused children tend to selectively attend to threat-related and aggressive

stimuli and tend to respond aggressively in situations involving a perceived threat or ambiguous social stimuli (Dodge, Lochman, Harnish, Bates, & Pettit, 1995; Dodge, Pettit, Bates, & Valente 1997; Pollak & Tolley-Shell, 2003; Price & Glad, 2003; Shields & Cicchetti, 1998).

A general lack of behavioral differences consistent with findings in both the primate and human literature may be attributable to the stimulus utilized during the Approach/Avoidance task. It is possible that the pig in the Approach/Avoidance test did not function as a highly aversive stimulus, which may be necessary to detect differences between maltreated and control subjects in fearful, aggressive, and/or anxiety-related behavior. These behavioral responses, which are expected during the Approach/Avoidance test, are emotion-related and have been demonstrated to be context dependent in both nonhuman primates and children (Bethea et al., 2004; Buss, Davidson, Kalin & Goldsmith, 2004; Cichetti & Rogosch, 2001a; Grand et al., 2005; Machado et al., 2009; Meunier et al., 1999; Williamson et al., 2003). For example, the stare condition of a Human Intruder test, a highly threatening social stimulus, elicits these behavioral responses both in rhesus infants in general (Kalin & Shelton, 1989) and significantly more in abused subjects when compared to controls (Grand et al., in preparation). However, these emotion-related behavioral responses were not significantly exhibited or exhibited at all during the profile condition (a less threatening social stimulus) of a Human Intruder test and conditions of an Approach/Avoidance test involving neutral or fear-evoking stimuli (Kalin & Shelton, 1989; Grand et al., 2005). Future studies utilizing the Approach/Avoidance test with nonhuman primates to examine maltreatment-related alterations in behavioral responses to novel objects may want to utilize more ecologically relevant aversive stimuli for the fear-evoking conditions of the test.

CSF Measures

As predicted, CSF concentrations of CRF, MHPG, 5-HIAA, and HVA correlated with behavior observed in the social compound and during the Approach/Avoidance test. In the social group, overall anxiety-related behavior and frequency of scratching, a reliable marker of anxiety in rhesus monkeys (Maestripieri, 1993), were positively correlated with levels of CRF and MHPG (with outliers removed) respectively. Individuals with higher CSF concentrations exhibited a higher frequency of anxiety-related behavior while individuals with higher MHPG concentrations more frequently scratched themselves.

Similarly, CRF and MHPG were correlated with anxiety-related behaviors during the Approach/Avoidant test. CRF was positively correlated with percentage of time spent inspecting the pig, indicative of vigilance and consistent with CRF stimulated heightened vigilance associated with HPA axis activation during a stress response (Gunnar & Vasquez, 2006), and displacement/self-directed behavior. Displacement/self-directed behavior in this study included self-groom, yawn, scratch, and body shake, which have been demonstrated as reliable indices of anxiety (Crawley et al., 1985; Schino, Perretta, Taglioni, Monaco, & Troisi, 1996; Troisi, 2002). MHPG was positively correlated with fearful/anxiety behavior and percentage of time spent manipulating the pig. These findings are consistent with the human literature which indicates that increased noradrenergic activity is associated with anxiety disorders (LaPrairie, Heim, & Nemeroff, 2010; Heim & Nemeroff, 2001) and studies of rhesus macaques which demonstrated that rates of anxious behavior were correlated with elevated levels of basal morning cortisol (McCormack et al., 2003).

Although not correlated with behavioral responses exhibited during the Approach/Avoidance test, CSF concentrations of HVA and 5-HIAA were correlated with

subjects' behavior directed toward infants in the social group. Levels of HVA and 5-HIAA were negatively correlated with duration of contact and frequency of affiliative behavior such that individuals with lower levels of HVA and 5-HIAA exhibited more affiliative behavior and longer durations of contact with infants. The finding that individuals with lower levels of 5-HIAA exhibit more affiliative behavior is contradictory to other studies of rhesus macaques which demonstrated that individuals with lower 5-HIAA concentrations exhibited reduced rates of social interaction (Higley et al., 1996). However, this contradiction could be due to methodological differences between the two studies; subjects in Higley and colleagues' study were either reared by their mothers or peer-reared and underwent a series social separations, a different type of early adversity than maternal maltreatment. Levels of 5-HIAA also were negatively associated with noncontact aggression and overall agonistic behavior which is consistent with the primate and human literature indicating increased levels of aggression associated with lower levels of 5-HIAA and deficits in serotonergic function (Higley, Mehlman, et al., 1992; Higley et al., 1996; Maestripieri, McCormack et al., 2006; Manji, Drevets, & Charney, 2001; Manuck et al., 1998; McCormack et al., 2009; Sanchez et al., 2007).

The prediction that maltreated subjects would have higher CSF concentrations of CRF but lower concentrations of MHPG, 5-HIAA, and HVA was not supported by the data. There were no significant group, sex, or group by sex differences between subjects on any of these measures. Other studies of rhesus macaques, which examined the developmental effects of early maternal abuse in biologically and foster raised infants, have also failed to find significant differences in CSF concentrations of 5-HIAA, HVA, and MHPG between abused and control subjects (Maestripieri, Lindell, et al., 2006; Maestripieri, McCormack et al., 2006). In both of these cases, maternal rejection rather than abuse was a better predictor of CSF concentrations of

5-HIAA and MHPG. However, no dimension of early maternal care predicted or correlated with CSF concentrations of 5-HIAA, MHPG, HVA, or CRF in this study. The findings from this study suggest early maternal care, particularly early maltreatment, does not result in long-term alterations in the synthesis and turnover of CRF, 5-HIAA, MHPG, or HVA. However, these findings should be interpreted with caution and need to be replicated and confirmed before definitive conclusions can be drawn since the CSF samples were obtained from a small sample with unequal sample sizes for the different group assignments.

The lack of maltreatment-related and maternal care related alterations in synthesis and turnover of CRF and monoamine metabolites but the presence of correlations between these measures and behavior in this study suggests that (i) as Maetripieri and colleagues (2006) argued, levels of HVA and MHPG may be more dependent on genetic inheritance and individual temperament than early maternal care and (ii) the associations between CRF, 5-HIAA, and early adverse care are complex. Future studies of maltreatment in macaques should examine the interaction between early maternal care and offspring temperament/personality on behavioral and neurochemical outcomes (Higley, Hasert, Suomi, & Linnoila, 1991).

Dimensions of Early Maternal Care as Predictors of Behavior

According to Bowlby's (1969) attachment theory, infants develop a sense of security and safety through attachment to their primary caregivers. Interactions with primary caregivers, particularly the caregiver's responses to the infant (i.e., parenting style), facilitate an infant's development of cognitive schemas which in turn enable the infant to regulate emotions and behavior (Bowlby, 1969). Variations in parenting style, which differentially influence the behavioral and physiological development of the offspring, have been observed in both human and animal studies. For example, Zahn-Waxler, Klimes-Dougan, and Slattery (2000) identified

risk factors for internalizing problems in children and adolescence, including lack of parental warmth, poor parental support, and parental hostility and rejection. In studies of rhesus macaques, variations in maternal parenting styles have been categorized as either 'rejecting/anxious' (characteristic of maltreating rhesus mothers) or 'protective/nurturing'. In these studies both dimensions of maternal care have been correlated with different behavioral outcomes in macaque offspring (Maestripieri, Lindell, et al., 2006; Maestripieri, McCormack et al., 2006; McCormack et al., 2006). McCormack and colleagues (2006) found that maternal abusive behavior, characteristic of rejecting/anxious maternal care, correlated with delayed independence in abused offspring.

In the present study, measures of behavior were related to specific dimensions of maternal care experienced during the first 3 months of life. In the social group, both frequency of play solicits and overall affiliative behavior directed toward juveniles were predicted by specific dimensions of maternal care. Average maternal protectiveness and responsiveness during the first 3 months of life were predictive of affiliative behavior toward juveniles such that individuals who had more protective or responsive mothers exhibited more affiliative behavior toward their peers at 18 months of age. Alternatively, average maternal irritability and average rates of maternal abuse were predictive of affiliative behavior toward juveniles such that individuals with more irritable or abusive mothers exhibited less affiliative behavior toward their peers. Average maternal responsiveness, irritability, and abuse were predictive of frequency of play solicits of peers in the same manner while average maternal security was positively correlated with frequency of play solicits. Frequency of avoidance of infants, was positively correlated with average maternal protectiveness, security, and responsiveness but was negatively correlated with average rates of maternal irritability and abuse.

Two dimensions of maternal care, responsiveness and irritability, also predicted behavioral responses during the Approach/Avoidance test. Average maternal responsiveness and irritability were predictive of percentage of time spent inspecting the pig. Subjects with more responsive mothers spent more time inspecting the pig while subjects with more irritable mothers spent less time inspecting the pig. Similarly percentage of time inspecting the pig was negatively correlated with average rates of rejection. Visually inspecting the pig may be the more appropriate or adaptive response to the pig in this particular context.

Altogether, these results suggest that, consistent with other studies of nonhuman primates, 'protective/nurturing' dimensions of maternal care are correlated with positive behavioral outcomes while 'rejecting/anxious' dimensions of maternal care are correlated with negative behavioral outcomes (Maestripieri, Lindell, et al., 2006; Maestripieri, McCormack et al., 2006; McCormack et al., 2006). Thus, an infant's early interactions (over the first 3 months of life) with their mother likely influenced their behavior and behavioral responses to stressors later in life.

Conclusion

This study provided evidence that maltreatment influenced rhesus social behavior and behavioral responses to stressors, namely affiliative and vigilant behavior. Additionally, there were significant correlations between CSF measures and behavior, indicating that genetic inheritance and individual temperament may play a larger role than originally predicted. Significant correlations between CSF measures and dimensions of maternal care and behavior indicate that early interactions with caregivers may influence the development of behavior and responses to stressors. However, there was no evidence that early maternal maltreatment leads to long-term neurochemical alterations in abused individuals.

It remains unclear whether the failure to find certain maltreatment-related differences in behavioral and neurochemical measures is due to the influence of other factors, such as resilience or temperament, an inadequate sample size (particularly for the CSF measures where the different groups had unequal and small sample sizes) or because maltreatment truly has little to no long-term effects in neurochemical development in rhesus monkeys. Future research should focus in more detail on specific characteristics of early maternal care and individual temperament to tease apart how these factors influence behavioral and neurochemical development in rhesus macaques. Future studies, using a cross-fostering design, should examine potential factors, such as quality of peer relations and resilience, which may buffer maltreated individuals against the negative developmental outcomes associated with experiencing early adverse care.

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APPENDIX A

LITERATURE REVIEW

Early adverse care, particularly childhood maltreatment in the form of abuse and/or neglect, is a serious public health problem in our society (Hussey, Chang, & Kotch, 2005; Sanchez & Pollack, 2009). Childhood maltreatment has been strongly implicated in the development of behavioral problems related to aggression and impulsivity as well as psychiatric disorders, predominantly mood, anxiety, and addictive disorders (Heim & Binder, 2012; Heim & Nemeroff, 2001; Kreppner, O'Connor, & Rutter, 2001; Pesonen & Räikkönen, 2012; Rogosch & Cicchetti, 1994; Sanchez, 2006; Sanchez, Ladd, & Plotsky, 2001). Additionally, childhood maltreatment has been associated with impairments in social competence, regulation of emotions, and cognition (Alessandri, 1991; Hart, Gunnar, & Cicchetti, 1995; Loman & Gunnar, 2010; Pesonen & Räikkönen, 2012; Rogosch, Cicchetti, & Aber, 1995; Sanchez & Pollak, 2009). In order to better understand the developmental consequences of early adverse care, research has focused on identifying the neurobiological mechanisms underlying the behavioral changes associated with childhood maltreatment.

Nonhuman Primate Model of Early Adverse Care

Animal models are frequently utilized in studies examining the developmental consequences of early life stress, which includes adverse care. Infant maltreatment has been reported to spontaneously occur in nonhuman primates, the closest animal models of childhood maltreatment in humans, in both captive and wild settings (Brent, Koban, & Ramirez, 2002; Johnson, Kamilaris, Calogero, Gold, & Chousos, 1996; Maestripieri, 1999, 1998; Maestripieri &

Carroll, 1998a; Maestripieri, Wallen, & Carroll, 1997a,b; Sanchez, 2006; Sanchez & Pollak, 2009; Sanchez, McCormack, & Maestripieri, 2010; Troisi & D'Amato, 1983; Troisi, D'Amato, Fuccillo, & Scucchi, 1982). In studies of early adverse care, rhesus macaques (*Macaca mulatta*) are the most commonly investigated nonhuman primate species (Sanchez, 2006). Similar to the rate of childhood maltreatment in humans, approximately 2-10% of rhesus infants are abused and/or neglected by their mothers (Maestripieri, 1999, 1998; Maestripieri & Carroll, 1998a,b; Maestripieri, Jovanovic, & Gouzoules, 2000). In these cases, physical maternal abuse is operationally defined as violent, infant-directed behavior including the mother crushing, throwing, dragging, and stepping/sitting on the infant (Maestripieri, 1998; Troisi & D'Amato, 1984). This atypical behavior causes significant infant distress, in extreme cases leading to serious physical injury or death. This abusive behavior also is distinct from other aggressive behavior which occurs during weaning-related conflicts, such as bites or slaps, and other behavior which is a part of the specie's aggressive repertoire. Maternal abuse has been observed to occur most frequently during the first 3 months of life with the highest rates of abuse occurring during the first and second months of life, is repeated with successive offspring, and is more prevalent in some matrilines indicating intergenerational transmission of maternal abuse (Maestripieri, 2005, 1999; Maestripieri, Tomaszycki, & Carroll, 1999; McCormack, Sanchez, Bardi, & Maestripieri, 2006; Sanchez, 2006).

In addition to exhibiting abusive behavior, approximately 70% of abusive mothers exhibit comorbid high rates of infant rejection very early after birth (Maestripieri, 1998; Maestripieri & Carroll, 1998a,b; McCormack et al., 2006; Sanchez 2006; Sanchez & Pollak, 2009; Sanchez et al., 2010). Infant rejection is operationally defined as the mother preventing contact or infant access to her nipple by holding the infant at a distance with an arm, passively blocking her chest

with an arm, or twisting her torso away from the infant (McCormack et al., 2006). The spontaneous occurrence of infant maltreatment in rhesus monkeys, in addition to its similarity to childhood maltreatment in humans, provides an opportunity to experimentally study the long-term, developmental effects of maternal maltreatment.

The Impact of Maternal Maltreatment on Behavior

Studies of early adverse care in humans indicate that the behavioral outcomes associated with experiencing childhood maltreatment often vary depending on the type of maltreatment the child experiences. These behavioral outcomes are often referred to as either internalizing problems, such as social withdrawal and anxiety, or externalizing problems, such as aggression and impulsivity (Bolger & Patterson, 2001; Cichetti & Rogosch, 2001a). Internalizing symptoms are often reported in children who experience neglect, physical abuse, or sexual abuse and in adult survivors of neglect, physical abuse, and sexual abuse (Bolger & Patterson, 2001; Keiley, Howe, Dodge, Bates, & Pettit, 2001; Manly, Kim, Rogosch, & Cicchetti, 2001; Young, Abelson, Curtis, & Nesse, 1997). Bolger and Patterson (2001) found that neglected and sexually abused children exhibited significantly higher levels of internalizing problems, especially if the child experienced both types of maltreatment, related to higher levels of perceived external control (i.e., outcomes in important life events are controlled by some unknown outside factor). In comparison to non-abused children and children who had experienced maltreatment later in life (6 to 9 years of age), Keiley and colleagues (2001) found that children who experienced maltreatment before age 5 exhibited more internalizing problems. In an adult population of anxiety and mood disorder patients, on average 25% of female patients and 16% of male patients experienced some form of childhood maltreatment (e.g., emotional abuse, physical abuse, or

sexual abuse). Additionally, severity and onset of psychiatric symptoms in these patients were associated with childhood abuse, particularly sexual abuse (Young et al., 1997).

Other studies have reported more externalizing symptoms in maltreated children and/or a combination of externalizing and internalizing symptoms (Cicchetti & Rogosch, 2001a; Cohen, Brown, & Smales, 2001; Manly et al., 2001). For example, in addition to reporting increased levels of internalizing symptoms in abused children, Keily and colleagues (2001) found that abused children were consistently rated higher by teachers as exhibiting both increased levels of internalizing and externalizing problems. When examining externalizing symptoms alone, abused and neglected boys in particular are reported as having greater incidences of externalizing problems, including increased aggression, violence, impulsivity, and delinquency (Manly et al., 2001; Stouthamer-Loeber, Loeber, Horrish, & Wei, 2001), possibly associated with deficits in threat perception. Studies have demonstrated that abused children tend to selectively attend to threat-related and aggressive stimuli and tend to respond aggressively in situations involving a perceived threat or ambiguous social stimuli (Dodge, Lochman, Harnish, Bates, & Pettit, 1995; Dodge, Pettit, Bates, & Valente 1997; Pollak & Tolley-Shell, 2003; Price & Glad, 2003; Shields & Cicchetti, 1998).

Studies of abuse and rejection in rhesus monkeys have demonstrated that experiencing maternal maltreatment in infancy leads to socioemotional alterations similar to those observed in maltreated children (Grand, McCormack, Maestripieri, & Sanchez, 2005; McCormack, Newman, Higley, Maestripieri, & Sanchez, 2009; McCormack et al, 2006). McCormack and colleagues (2006) found that abused infants exhibited higher rates of screams and tantrums (i.e., were more emotionally reactive), broke contact with their mother less often, and spent a greater proportion of time in solitary play than controls during the first 6 months of life. Additionally, multi-

dimensional scaling analyses revealed a general pattern of delayed social development and increased levels of distress and dependency in these abused infants when compared to controls (McCormack et al., 2006). When tested for behavioral reactivity to novel stimuli, abused rhesus juveniles (approximately 24-28 months old) exhibited context-dependent alterations in behavior in comparison to controls (Grand et al., 2005). In this study, in the presence of a Human Intruder, abused juveniles displayed more anxious and fearful behavior, consistent with internalizing symptoms reported in studies of neglected, physically abused and sexually abused children, particularly females (Bolger & Patterson, 2001; Keiley et al., 2001; Manly et al., 2001; Young et al., 1997). However, abused juveniles were more aggressive/impulsive and less fearful in the presence of novel objects (e.g., shorter latency to investigate fear-evoking objects, more exploration when presented with neutral objects) which also is consistent with externalizing symptoms reported in maltreated children, particularly males (Cicchetti & Rogosch, 2001a; Kaplow & Widom, 2007; Manly et al., 2001; Sanchez & Pollak, 2009). Grand and colleagues point out that these behavioral alterations in abused versus control subjects may reflect impairments in threat-assessment and/or increased emotional reactivity that manifests as different behavioral responses in different contexts (e.g., an individual may have an underlying state of fear that can manifest as increased freezing in one context, but increased aggression in a different context).

Maltreatment-Related Alterations of Stress- and Threat-Response Systems

Preclinical studies of rhesus monkeys and other animal models have indicated that stress- and threat-response systems are particularly sensitive to early adverse care (Sanchez et al., 2001). It has been hypothesized that maltreatment-related alterations in these systems may underlie the socioemotional and cognitive alterations observed in maltreated children and animal models. In

this early life stress model, chronic over-activation of these systems as a result of experiencing childhood maltreatment may alter the development of neural systems involved in the regulation of attention and emotion. Maltreatment-related developmental alterations in these stress- and threat-response neural systems then result in impairments in the regulation of attention and emotions (Loman & Gunnar, 2010). For example, functioning of the hypothalamic-pituitary-adrenal (HPA) axis, which is a main neuroendocrine stress response system that also plays an important role in emotion regulation, is altered by the experience of early life stress in the form of adverse early care. Studies of both humans and animal models have demonstrated that the experience of early adverse care results in dysregulation of the HPA axis, which may include both hypo- and hyper-functioning (for reviews see Bruce, Gunnar, Pears, & Fisher, in press; Doom Cicchetti, Rogosch, & Dackis, in press; Gershon Sudheimer, Tirouvanziam, Williams & O'Hara, 2013; Gunnar & Herrera, 2007; Sanchez, 2006; Sanchez, et al., 2010; Sanchez & Pollak, 2009; Tarullo & Gunnar, 2006).

Maltreatment-related Dysregulation of the HPA Axis

Studies examining HPA axis functioning in maltreated children have reported alterations in basal and diurnal cortisol levels as well as HPA responsiveness to stressors and pharmacological challenges. Maltreated children exhibit elevated basal and diurnal cortisol levels in some instances, although there also are reports of reduced basal cortisol levels and flattened but low diurnal cortisol activity (for reviews see Bruce et al., in press; Doom et al., in press; Gershon et al., 2013; Gunnar & Herrera, 2007; Gunnar & Quevedo, 2007; Sanchez, 2006; Tarullo & Gunnar, 2006). For example, Cicchetti and Rogosch (2001b) found that, in comparison to nonmaltreated children, children who experienced physical and sexual abuse exhibited higher morning cortisol levels and that children who experienced multiple types of

abuse exhibited elevated cortisol levels both in the morning and afternoon. However, they also found that a subgroup of children who experienced physical abuse exhibited lower morning cortisol levels as well as a blunted diurnal cortisol rhythm in comparison to nonmaltreated children. It has been suggested that this variability may be dependent on specific aspects of early adverse care, such as the type of adverse care (e.g., physical abuse, sexual abuse, neglect, etc.) or its developmental timing (Heim, Ehlert, & Hellhammer, 2000; Miller, Chen, & Zhou, 2007; Sánchez et al. 2001), or ongoing trauma/maltreatment at the time of assessments. Low morning cortisol levels have been associated with severe physical neglect whereas high morning cortisol levels have been associated with severe emotional maltreatment (Bruce et al., in press) although it also can be a consequence of adaptive downregulation of HPA axis activity after overactivity (Fries, Hesse, Hellhammer, & Hellhammer, 2005).

Hypo- and hyper-activity in HPA responsiveness to challenges also has been reported in maltreated children (Bugental, Martorell, & Barraza, 2003; Carrion et al., 2002; De Bellis, et al., 1994; Hart et al., 1995; Kaufman et al., 1997; MacMillan et al., 2009; Rao, Hammen, Ortiz, Chen, & Poland, 2008). Depressed adolescents, who also had experienced early adversity and high levels of chronic stress during adolescence, exhibited higher and more prolonged cortisol responses to a standardized psychosocial stressor (the Trier Social Stress Test) modified for use with children (Rao et al., 2008). In two other studies, maltreated female adolescents, in comparison to control females, and maltreated/bullied children, in comparison to controls, exhibited a blunted cortisol response to the same and/or similar psychosocial stressors (MacMillan et al., 2009; Ouellet-Morin et al., 2011). Additionally, sexually abused adolescent females, who had a greater incidence of suicidal ideation, suicide attempts, and dysthymia,

exhibited attenuated adrenocorticotropin hormone (ACTH) responses to corticotropin-releasing hormone (CRH) challenge (De Bellis et al., 1994).

These alterations in HPA responsiveness to challenges appear to persist into adulthood (for reviews see Doom et al., in press; Gershon et al., 2013; Tarullo & Gunnar, 2006). Heim and colleagues (2002) found that women who had a history of childhood abuse, regardless of whether they were currently suffering from depression, exhibited increased ACTH reactivity to a psychosocial stressor. Similarly, the same group demonstrated that men with a history of childhood abuse, again regardless of current depression status, exhibited increased cortisol and ACTH reactivity to dexamethasone/CRF challenge (Heim, Mletzko, Purselle, Musselman, & Nemeroff, 2008). Other studies have reported attenuated cortisol and ACTH responses to psychosocial stress in adults with a history of childhood maltreatment but no current psychopathology (Carpenter et al., 2007) as well as diminished afternoon cortisol levels in females with posttraumatic stress disorder (PTSD) and a history of abuse (Bremner, Vermetten, & Kelley, 2007).

Studies of nonhuman primates have demonstrated similar alterations in HPA axis functioning as a result of early adverse care (e.g., poor rearing conditions, maternal and peer separations, and maltreatment) (for reviews see Sanchez, 2006; Sanchez et al., 2010; Sanchez & Pollak, 2009). Elevated basal cortisol levels in infancy, followed by lower than normal cortisol levels, including flattened diurnal cortisol rhythms, during the juvenile period have been reported in studies of infant maltreatment (Koch, McCormack, Sanchez, & Maestripieri, in press; McCormack et al., 2003) and maternal separations, particularly in females (Sanchez et al., 2005). Similarly, elevated basal cortisol levels also have been reported in peer-reared infants and juveniles in comparison to mother-reared individuals (Champoux, Coe, Schanberg, Kuhn, &

Suomi, 1989; Higley, Suomi, & Linnoila, 1992). Other studies comparing the effects of rearing conditions (e.g., peer-reared, mother-reared, isolation-reared) on basal HPA axis functioning in infancy and the juvenile period found no differences in basal cortisol levels when comparing peer-reared to mother-reared or isolation-reared individuals (Clarke, 1993; Meyer & Bowman, 1972) and lower basal cortisol and ACTH levels when comparing peer-reared to mother-reared individuals (Clarke 1993; Shannon, Champoux, & Suomi, 1998).

Alterations in HPA responsiveness to stressors and pharmacologic challenges also have been reported in these studies. Koch and colleagues (in press) found that, in addition to having elevated basal cortisol levels which attenuate later in life, abused individuals also exhibited higher cortisol responses to stressors (a novel environment test) during the first year of life but not later in life. Additionally, at one year of age, abused females exhibited higher stress responses when compared to controls but abused males exhibited lower stress responses when compared to control males (Koch et al., in press). These findings indicate a short-term effect of early abuse on HPA axis response to stress which differs for male and females, consistent with findings in other studies of rhesus macaques (see McCormack et al., 2009). Furthermore, abused rhesus infants exhibit greater cortisol but blunted ACTH responses to CRH challenge during the first 3 years of life (Sanchez et al., 2010). Similar alterations in HPA responsiveness to CRH challenge have been reported in young adult common marmosets that had experienced maltreatment early in life (Johnson et al., 1996).

Neurobiology Underpinning Maltreatment-Related Socioemotional Alterations

The focus of recent studies, including the present one, has been to better characterize and understand the neurobiology underpinning maltreatment-related socioemotional and cognitive alterations by examining maltreatment-related developmental changes in neurochemical

synthesis and turnover. A number of studies have examined the role of hypothalamic and extrahypothalamic corticotropin-releasing factor (CRF) systems and of the brain monoaminergic systems in stress reactivity and psychopathology. CRF regulates behavioral, autonomic (sympathetic) and endocrine responses to stressors, including mediation of the HPA axis and sympathetic-adrenomedullary (SAM) activations during the stress response, as well as some of the behavioral effects are mediated via effects on brain monoaminergic systems (Gunnar & Herrara, 2007; Gunnar & Quevedo, 2007; Gutman & Nemeroff, 2003; Sanchez & Pollak, 2009). The monoaminergic systems of particular interest include the brain serotonergic system, which is involved in emotion regulation and impulse control, the brain noradrenergic system, which is involved in arousal regulation and threat-response, and the brain dopaminergic system, which is involved in reward-related behavior, motivation, behavioral inhibition, and prefrontal cognitive functions (Higley et al., 1992, 1996; Kaufman, Plotsky, Nemeroff, & Charney, 2000; Maestripieri, McCormack, et al., 2006; Sanchez et al., 2001). Alterations in each of these systems have been implicated in different forms of psychopathology. Hypersecretion of CRF is associated with depression, suicide, and post-traumatic stress disorder (for reviews see Gershon et al., 2013; Heim & Binder, 2012). Reduced serotonergic function is associated with mood disorders as well as increased anxiety, aggression, and impulsivity (Maestripieri, McCormack et al., 2006; Manji, Drevets, & Charney, 2001; Manuck et al., 1998; McCormack et al., 2009; Sanchez et al., 2007). Increased noradrenergic activity also is associated with depression, as well as anxiety disorders (LaPrairie, Heim, & Nemeroff, 2010; Heim & Nemeroff, 2001). Over-activity of the brain dopaminergic system has been implicated in schizophrenia and addictive disorders (Moghaddam, 2002; Skelton, Weiss, & Bradley, 2010). Investigation of alterations in levels of neurotransmitters and neuropeptides in these systems in response to childhood

maltreatment is important in understanding how the experience of early adverse care may sensitize these systems to stress and the development of later psychopathologies.

The Impact of Early Adverse Care on Neuropeptides and Neurotransmitters Synthesis and Turnover

To assess how early adverse care influences neuropeptide and neurotransmitter synthesis and turnover, researchers often examine cerebrospinal fluid (CSF) concentrations of CRF and monoamine metabolites, which are used as indices of brain CRF activity and monoamine production and turnover (Higley, et al., 1992; Shannon et al., 2005; Pryce et al., 2005; Maestripieri, McCormack et al., 2006). These monoamine metabolites include 5-hydroxyindoleacetic acid (5-HIAA), a serotonin metabolite, 3-methoxy-4-hydroxyphenylgycol (MHPG), a metabolite of norepinephrine, and homovanillic acid (HVA), a dopamine metabolite (for a review see Pryce et al., 2005). Research has demonstrated that alterations in CSF concentrations of CRF and these monoamine metabolites are associated with the occurrence of psychopathology and impairments in both human and animal models. For example, low CSF concentrations of 5-HIAA in humans have been associated with increased impulsivity, aggression, emotional reactivity, and anxiety and mood disorders, including major depressive disorders (MDD) and an increased vulnerability of acting on suicidal impulses in individuals suffering from MDD (Berman & Coccaro, 1998; Manji et al, 2001; Mann, Oquendo, Underwood, & Arango 2009). In nonhuman primates, low CSF concentrations of 5-HIAA have been associated with increased anxiety, risk-taking, and social impulsivity as well as severe aggression (Fairbanks, Melega, Jorgensen, Kaplan, & McGuire, 2001; Maestripieri, McCormack, et al., 2006; Melhman et al., 1994; McCormack et al., 2009).

While studies of neurochemical alterations in humans have focused on dysregulation of the HPA axis, studies specifically investigating the impact of early adverse care on neurotransmitter synthesis and turnover in rhesus monkeys have revealed some maltreatment-related alterations in CSF concentrations of monoamine metabolites. In one study, lower CSF concentrations of 5-HIAA and HVA at 1, 2, and 3 years of age were associated with experiencing higher levels of maternal rejection during the first 6 months of life, regardless of whether these infants were raised by their biological mothers or were cross-fostered to unrelated females (Maestripieri, Lindell, et al., 2006). Furthermore, abused subjects in this study who became abusive mothers continued to have lower 5-HIAA concentrations, indicating a potential role for maltreatment-related dysregulation of the serotonergic system in the intergenerational transmission of abusive behavior in macaques (Maestripieri, Lindell, et al., 2006).

An additional study by Maestripieri and colleagues (2006) also found that experiencing early maternal rejection is associated with lower CSF concentrations of 5-HIAA regardless of whether these individuals were raised by their biological mothers or were cross-fostered to unrelated females. Juveniles that experienced high levels of rejection and had lower CSF 5-HIAA concentrations engaged in solitary play more frequently and exhibited high rates of scratching, indicative of anxiety, at 2 years of age in comparison to those that experienced low levels of maternal rejection in early infancy. The association between CSF concentrations of 5-HIAA and maternal rejection were similar between cross-fostered and non-cross-fostered subjects, suggesting that this association was unlikely due to differences in genetic inheritance (Maestripieri, McCormack, et al., 2006). Unlike 5-HIAA, CSF concentrations of HVA and MHPG were not significantly affected by exposure to maternal rejection in the first 6 months of life. There was, however, a weak association between exposure to maternal rejection in the first 6

months of life and lower CSF concentrations of MHPG at the second year of life as lower concentrations of MHPG were associated with increased frequency of solitary play and avoidance behavior at 2 years of age. Since exposure to maternal rejection predicts solitary play but not CSF concentrations of MHPG, Maestripieri and colleagues suggest that socially phobic behavior and fearfulness may be more dependent on genetic inheritance and only weakly affected by early adverse experience (i.e., individuals with low CSF concentrations of MHPG may be more socially phobic and fearful in general, regardless of early experience).

In other recent studies examining the relationship between early maltreatment, serotonergic function, behavioral responses to stress, and emotional reactivity of rhesus monkeys, although there was no effect of maternal abuse on CSF concentrations of 5-HIAA at 24 to 28 months of age, there was a strong effect of rejection by the mother (Sanchez et al., 2007). In both papers, lower 5-HIAA concentrations in the juveniles were associated with increased levels of anxiety in the social group (Sanchez et al., 2007) and behavioral responses to novel, fear-evoking objects indicative of fear and behavioral inhibition as well as behavioral responses to novel, neutral objects indicative of vigilance (Grand et al., in preparation). Sanchez and colleagues (2007) also found that lower 5-HIAA concentrations were associated with stress-related increases in p38 mitogen-activated protein kinase (MAPK) activity, likely as a result of deficits in serotonin metabolism and increased serotonin transporter expression/activity caused by activation of p38 MAPK signaling pathways, both of which decrease synaptic availability of serotonin. Similarly, Bethea, Centeno, and Cameron (2008) found evidence that early life stress causes deficits in serotonin synthesis and autoreceptors in the raphe nuclei related to decreased gene expression of mRNAs crucial for normal serotonin function. Although these studies demonstrate that there are some associations between early adverse care, neurotransmitter

synthesis and turnover, particularly in the serotonergic system, and behavior, the neurobiological mechanisms underlying maltreatment-related behavioral alterations are only beginning to be understood.

Conclusions

From this review it is clear that experiencing early adverse care impacts and/or is associated with a variety of negative behavioral and neuroendocrine outcomes in humans and nonhuman primates. Additionally, the behavioral and neuroendocrine alterations appear to be associated with each other, making the relationship between maltreatment and behavioral and neuroendocrine development more complex. The underlying mechanisms by which maltreatment influences these outcomes and the nature of the relationship between behavioral and neuroendocrine outcomes are only beginning to be understood. Continued research which experimentally examines the relationships and variables outlined above are necessary in order to gain a clearer understanding of the role that experiencing early adverse care plays in an individual's socioemotional, cognitive, physiological, and neural development.

APPENDIX B

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APPENDIX C

LABORATORY PROTECTIVENESS SCALE

Maternal and Infant Rhesus Rating Scales (after real time observation)

For the below items, rate HOW OFTEN each behavior occurred during the observation period.
For items that refer to threatening events (indicated with *****), please rate "N/A" if no such event occurred. "Threatening" is operationalized as (1) non-social events that agitate the group (overhead objects, the vets), and (2) social threats to the infant (attack/threat, kidnap, scuffles that break out nearby infant, aggressive animal nearby, large fights in the group).

Use the following scale for all questions (except for those related to Abuse/Rejection severity):

N/A	**1**	**2**	**3**	**4**	**5**
	Never	**Rarely**	**Sometimes**	**Often**	**Always or almost all time**
	(0%)	**(1-25%)**	**(26-50%)**	**(51-75%)**	**(76-100%)**

MATERNAL BEHAVIOR dimensions

1) Mother makes herself available when infant approaches her (opens body to infant, does not walk away or block nipple)

2) Mother responds to infant's signals (e.g. distress) and bids for contact/grooming.
[Note: bids for contact may be screams/tantrums because mother left it]

3) Mother adjusts caretaking behavior based on infant's response (e.g. stops grooming if infant doesn't like it).

4) Mother comforts infant when distressed/upset/fearful.

5) Mother holds infant in ventrum right away when this is distressed and returns to mother for contact.

6) Mother is comfortable and relaxed when in contact with infant.
[Opposite: mother seems uncomfortable by infant's behaviors in ventrum, moves infant's position, jerks or shows other annoyed behaviors].

7) Mother appears distressed/annoyed by infant's demands.

8) Mother monitors infant when away from her.

9) Mother signals infant to follow when she moves away.

***** 10) During threatening events, mother makes/maintains contact with infant or prevents it from leaving.
[Note: if infant is away, mom knows exactly where it is.]

***** 11) Mother retrieves infant right away if it is attacked/threatened or emits distress calls

***** 12) Mother "guards" infant (restrains/cradles/draws it closer) when a potentially threatening animal walks by (adult male, high ranking/aggressive female).

***** 13) If infant is kidnapped, mother monitors the situation (follows kidnapper, makes bids for contact).

14) Mother allows infant to use her body to play, explore, climb from, etc.

15) Mother allows infant to explore its surroundings and/or play.

16) Mother allows infant to leave and return to her (refueling: e.g. play & return; explore & return)

17) Mother is inconsistent in responding to infant's needs or bids for contact/interaction
[1: very consistent; 5: very inconsistent]

18) Mother goes from cradling/caring for infant to abuse/rejection (or vice versa) without clear reason

19) Caretaking bouts are brief. Mother stops infant's care without clear reason (e.g. interruption, infant signal)

20) Mother uses only physical behaviors to control infant (e.g. restrains, punishes by biting), instead of using facial expressions or gestures (e.g. threat, lip smacking).

21) Mother punishes infant (bites, slaps) for minor negative behaviors.

22) Mother continues/repeats punishment even after infant stops negative behavior

ABUSE SEVERITY:

23) Rate the severity of physical abuse (based on your impression & infant's distress):

0	1	2	3	4	5
NA	Mild		Moderate		Severe

24) The abusive episodes lasted:

0	1	2	3	4	5
NA	Short (a few seconds)		30 seconds		Very long (>1 min)

25) Rate the infant's distress level during the abusive event:

0	1	2	3	4	5
N/A	No Distress		Moderate		Severe Distress

26) Was the infant injured as a result of the abuse? 0 – No 1 – Yes

IF YES, please describe the infant's injuries (add veterinary records, if applicable):

_____

_

REJECTION/NEGLECT SEVERITY:

27) Rate the intensity of rejections (based on your impression & infant's distress; for example: blocking chest passively is less intense than actively pushing/pulling infant off her body, sometimes followed by biting):

0	1	2	3	4	5
N/A	Mild		Moderate		Severe

28) Rate the infant's distress level during the rejection event:

0	1	2	3	4	5
N/A	No Distress		Moderate		Severe Distress

29) How long did the rejections bouts last:

0	1	2	3	4	5
NA	Short(a few seconds)		30 seconds		Very long (> 1 min)

INFANT BEHAVIOR

***** 30) During threatening events, infant approaches mother without hesitancy

***** 31) During threatening events, infant goes to an animal other than its mother

32) Infant has to search for mother before returning to her; unaware of her location.

33) Infant checks in with mother when away from mother (going back to her often; making visual contact)

34) Infant seems comfortable when on/around mother (playing/exploring around her, relaxed body posture)

35) Infant jerks/tantrums in response to proper maternal care (e.g. feeding, grooming, retrieving under threat)

36) Infant is hesitant/anxious to approach or contact mother

37) Infant becomes distressed when mother leaves (follows her with distress vocalizations).

38) Infant shows distress (screams, tantrums) in response to mother rejection

39) Infant is passive (i.e., not engaged in play/exploration, contact with mother, sleep, eating).

40) Infant actively chooses what to do (i.e., mom does not dictate the activity)

41) Infant plays roughly/aggressively with peers, willing to hurt/injure them. (Peers scream or retreat from play.)

42) Of the time infant played/explored, what % time did that happen >1 meter from mother?

43) Of the time infant played/explored, what % time did that happen within 1 meter of mother?

44) What % of time does infant spend in contact with animals other than mother (excluding play)?

APPENDIX D

ETHOGRAM OF RHESUS BEHAVIOR

at	attack	common definition
av	avoidance	turning or moving away in response to animal approach within 1 meter
bi	bite	common definition
bs	body shake	shaking fur like a wet dog
ch	chase	pursuit in agonistic context, not in play
co	contact	body contact initiated
c-	end contact	body contact terminated
cp	contact to prox	break contact into proximity. Mother-infant code only
vo	on ventrum	infant is in ventral contact, but not nipple contact
v-	off ventrum	ventral contact ends
do	on dorsal	infant is on dorsum
d-	off dorsal	dorsal contact ends
oc	other contact	code when infant is in any other form of contact than those listed above
o-	other ended	code when other contact ends
oo	infant coo	cooing by infant
cr	cradle	ventro-ventral contact with the other animals arms wrapped around infant
ce	end cradle	cradle terminated
dp	display	infant bounces up and down, shakes equipment.
dc	dorsal carry	mother carries infant on her back during the first month of life
ea	eating/drinking	animal is eating or drinking water
e-	end eating/drinking	
gi	gential inspec	inspection of another animal's genitals (to or from infant)
gs	groom solicit	presenting flank or rump to solicit grooming
fg	grimace	bared-teeth display
gm	groom	picking and spreading fur of other animal
g-	end groom	grooming termintated
ht	hit	mother slaps infant
hr	harass	pulls, drags, or hits another
kn	kidnap infant	other individual prevents infant from returning to mother (>1 min.). There must be obvious signs of distress on the part of the mother or the infant. Do not score behaviors during kidnap, other than kidnap scream.
k-	end kidnap	terminates kidnapping
ks	kidnap scream	infant screams during a kidnap
kc	kidnap coo	infant coos during a kidnap
lc	limb carry	mother carries infant on her limb, with infant parallel to her arm, clinging, during first month of life
ls	lipsmack	repeated lip movements
mn	mount	common definition
pa	passive	animal is sitting or standing passively, and does not fit into other categories
pe	end passive	
pr	present	animal orients hindquarters toward other with raised tail
px	prox	animal enters and stays within 1 ft diameter of a stationary animal. **Mother-infant code only**
pc	prox to contact	animal initiates contact after proximity was established. **Mother-inf only**
pl	prox to leave	animal leaves outside of proximity (not contact). **Mother-inf only**
rj	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

rs	restrain	actively prevent infant from breaking contact by pulling its leg or tail
sc	scratch	common definition (mother during first two months)
sm	infant scream	acute vocalization by infant, not coo's
sg	self-groom	picking and spreading one's fur
s-	end self-groom	self-groom terminated
sp	social play	rough and tumble, chase play with another animal
p-	end social play	social play terminated
ps	play solicit	attempt to play with another, without success
ip	solitary play	play with objects, or running, spinning, and gamboling
i-	end sol play	solitary play terminated
ta	tantrum	infant's body shakes, while infant geckers or screams
tr	threat	open mouth, stare with/without woofing
to	touch	other individual touches infant, or vice versa
vc	vocalize	vocalization of any kind, other than a scream, coo, or threat made by the infant, or towards the infant (ex: gurney, grunt)
ya	yawn	common definition (mother during first two months)

Abusive Behavior

dr	drag	drags infant while walking or running
cu	crush	pushes infant against the ground with hands
th	throw	throws infant ahead while walking or sitting
st	step on/sit on	steps or sits on infant
rg	rough grooming	holds infant into the ground, and pulls out infant's hair with force
ac	rough carry	carries infant with one arm away from her body, infant unable to cling

Behavior added 2007

fr	freeze	animal remains motionless, except for slow head movements for at least 3 seconds. Tense body posture. No vocalizations. Not crouch freeze. Hanging or on ground.
cf	crouch freeze	motionless, tense posture, with ventrum pressed down to ground for at least 3 seconds
ae	avert	turns away from stimulus, avoiding eye contact for at least 3 seconds. Not motionless or tense
wd	withdrawal	quick, jerky motion backwards after forward movement towards stimulus
ag	agitation	Rapid, jerky movements, shaking equipment violently, biting equipment, trying to escape when in cage
dl	depressive-like	head down, self-clutching, rocking.
so/se	stereotypies	repetitive, non-changing motor pattern (e.g., circling, pacing, rocking). It occurs 3 or more consecutive times
lo/le	locomotion	ambulation of 1 or more full steps at any speed. Includes jumping or dropping from equipment

New Behavior 2010

bj **body jerk** a quick, vertical jerking movement, different than a shake, of the mom (maybe a stunted quick shake possibly indicating she's annoyed); can be thought of as an adult version of a tantrum

sr **stretch rejection** an indirect way of removing the infant from mom's ventrum in which mom stretches her arms upward causing the infant to slip down her ventrum and/or off nipple

bp **back pat** common definition; infant on ventrum, mother pats back repeatedly in a quick motion. 2-3 quick pats =1 freq for this code.

New Identifier 2010

oi **other infant** refers to the new, younger sibling of current subject (most likely only applicable at 12 and 18 months)