COGNITIVE CONSEQUENCES OF MATERNAL MALTREATMENT IN JUVENILE RHESUS MONKEYS

by

DESIREE ISIS SHARPE

(Under the Direction of Irwin Bernstein and Mar Sanchez)

ABSTRACT

Childhood maltreatment is a form of early life stress associated with severe behavioral, socioemotional, and cognitive developmental outcomes. The present study used a translational animal model to evaluate specific cognitive consequences of maternal maltreatment. Newborn rhesus monkeys (*Macaca mulatta*; n = 36, 16 male, 20 female) were cross-fostered with multiparous mothers who previously had been identified as either maltreating or competent based on established measures of abuse and neglect. At 18 months of age, subjects participated in a series of cognitive tasks that assessed some prefrontal cortex (PFC)-mediated cognitive functions, including behavioral flexibility and inhibitory control (Object Retrieval Detour task, ORD), and working memory (Delayed-Non-Matching-to-Sample Session Unique, DNMS-SU). Maternal behavior data, including frequency of abuse, rejection, restraint, and scores on a maternal rating scale designed to address maternal behavior dimensions (Sensitivity/Responsivity, Protectiveness, Attachment, and Irritability) were collected, as well as CSF concentrations of the monoamine metabolites 5-HIAA, HVA, and MHPG, and a primary stress neuropeptide, CRF. Although there was no effect of experimental group, sex differences, or interactions on cognitive task performance or CSF monoamine metabolite concentrations and

CRF levels, we found significant correlations between dimensions of maternal care and performance on the ORD and DNMS-SU, as well as between CSF measures and cognitive task performance. On the ORD task, animals that experienced high rates of rejection were more likely to balk following an initial attempt on a trial, and monkeys who experienced more restraint and responsivity from their mothers exhibited increased Day 1 latencies. Furthermore, 5-HIAA, a serotonin metabolite, was correlated with barrier and perseverative reaches, indicating altered serotonergic ofPFC-mediated cognition in animals who perseverated. On the DNMS-SU task, animals that experienced more abuse and restraint committed more errors to criterion. Our results indicate that in addition to the continued investigation of neurochemical substrates of altered cognition in maltreated individuals, it is critical that we examine global dimensions of maternal maltreatment in order to better explore the qualities of maternal care that may have more impact on the behavioral, neurodevelopmental, and cognitive outcomes of maltreated monkeys than frequency rates of abuse and/or rejection alone.

INDEX WORDS: early life stress, monkey model, cognitive development, object retrieval detour, DNMS-SU, maternal maltreatment, *Macaca mulatta*, sex differences, 5-HTTLPR, CRF, 5-HIAA, MHPG, HVA

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DEDICATION

This dissertation is dedicated to my father, Robert Wayne Sharpe, who taught me that life is an attitude. Your wisdom, courage, strength, grace, and magic mean more to me than I can say. I think of you every day, with the purest love in my heart.

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CHAPTER 1

INTRODUCTION

Early social experiences, particularly maternal care, shape development in maltreated animals (Brent, Koban, & Ramirez, 2002; Harlow, 1974; Heim & Binder, 2012; Hersher, 1969; Janus, 1987; Lehmann & Feldman, 2000; Maestripieri & Carroll, 1988b; Maestripieri, Wallen, & Carroll, 1997a; McCormack, Sanchez, Bardi, & Maestripieri, 2006; Newell, 1967; Sanchez, Ladd, & Plotsky, 2001; Sanchez & Pollak, 2009; Scott, 1958; Seay & Harlow, 1965; Suomi, 1976). In humans, childhood maltreatment by parents is a devastating adverse experience that includes both abuse and neglect and can result in severe behavioral, emotional, and cognitive consequences (e.g., Beers & DeBellis, 2002; Carrey, Butter, Persinger, & Bialik, 1995). Abuse can be physical (i.e., hitting, kicking, burning; Sedlak & Broadhurst, 1996), sexual (any sexual activity with a child, including watching pornography or involving child in pornography; Garbarino & Garbarino, 1994), and/or emotional (i.e., verbal abuse, exposing child to domestic violence; Putnam, 2003). Neglect occurs when caretakers do not attend to the child's need for affection or emotional support, do not provide proper shelter or nutritional needs, allow regular school absences, and/or permit alcohol and drug use (English, Thompson, Graham, & Briggs, 2005). Victims of childhood abuse and neglect often display both internalizing psychiatric disorders, such as clinical depression, generalized anxiety disorder, post-traumatic stress disorder (PTSD), dissociative experiences, and heightened suicidality, as well as greater externalized maladaptive behavior, including episodic aggression, conduct disorder, attention deficit disorder (ADHD), substance abuse, and greater impulsivity (DeBellis, 2002; Hart & Rubia, 2012).

Abuse and neglect also can affect normal cognitive development. Maltreatment is associated with lower IQ scores and poor academic performance (Hart & Rubia, 2012), language and memory impairments (Bos, Fox, Charles, & Nelson III, 2009), and inhibitory control problems (see Pechtel & Pizzagalli, 2010, for a review). Executive function deficits can emerge in victims of maltreatment at an early age and continue into adulthood (Spann et al., 2012), but these impairments often are specific to particular aspects of cognitive function (e.g., Pears & Fisher, 2005; Pollak et al., 2010). For example, Pollak et al. (2010) found that while maltreated children (8 – 9 years of age) in their study performed as well as controls on some executive functions, such as rule acquisition and planning, they displayed working memory and inhibitory control problems. The results from Pollak et al. (2010) indicate the possibility that the effects of early life stress on cognitive function are not global, and instead affect specific neural circuits involved in different aspects of cognition.

The neurobiological mechanisms that underlie the effects on specific cognitive pathways, as well as their development throughout infant and juvenile sensitive periods, are not well understood. The dearth of knowledge regarding these processes is in part due to the challenges associated with prospective, longitudinal studies in human populations. Given that infant maltreatment also is reported in rhesus monkeys (e.g., Suomi, 1976; Maestripieri, 1998), this species provides a good primate animal model with a well-developed prefrontal cortex (Kojima & Goldman-Rakic, 1984) that can allow researchers to better explore how genetic factors, neuroendocrine function, neurochemical systems, and social behavior interact with early maternal care to affect juvenile cognitive function. The present study examined these processes using juvenile rhesus monkeys as a translational model for cognitive consequences associated with developmental changes in stress regulation systems seen in abused and neglected

individuals. As we continue to develop a better understanding of the neurobiological underpinnings of cognitive function and how early experiences, particularly maternal care, affect its development using the rhesus monkey model, we also can better identify potential alternatives of prevention, treatment, and protective factors for victims of abuse.

Neurobiological Substrates of Early Life Stress on PFC Structure and Function

From a neurobiological perspective, maltreatment is considered an extremely severe form of early life stress that impacts the sympathatic-adrenomedullary (SAM) and hypothalamic-pituitary-adrenal (HPA) stress neuroendocrine systems, as well as the monoaminergic neuromodulatory pathways involved in stress regulation (Gunnar & Herrara, 2007; Gunnar & Quevedo, 2007; Gutman & Nemeroff, 2003; Sanchez & Pollak, 2009). Stress activation of the SAM system in the peripheral nervous system (PNS) results in adrenal medullary release of norepinephrine and epinephrine, causing muscular vasodilation, increased heart rate (part of the "fight or flight" response), and enhanced energy mobilization. Subsequently, SAM activity in the central nervous system (CNS) enhances arousal, increases vigilance, and directs attention to potential stressors. HPA axis activity is much slower in its response to potentially threatening stimuli than the SAM system, with glucocorticoids taking several minutes for peak onset of release and binding. In contrast to peripherally-released epinephrine and norepinephrine in the SAM system, glucocorticoids can cross the blood-brain barrier and bind to both mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) in the CNS. Chronic, early life stress can result in long-term behavioral, neuroendocrine, and neurobiological consequences (Sanchez, 2006), including altered HPA function in the form of hyper- and hypocortisolemia (for reviews, see Gunnar & Herrera, 2007; Sanchez, 2006; Sanchez, et al., 2010; Sanchez & Pollak,

2009). Alterations in the SAM system and HPA axis are some of the potential mechanisms through which early life stress can affect cognitive development in primates.

In addition to the HPA axis and SAM system, stress both regulates and is regulated by certain monoaminergic neuromodulatory pathways, including the serotonergic system (5-HT; emotion regulation, impulsivity), noradrenergic projections (threat assessment), and dopaminergic pathways (appetitive behavior, behavioral inhibition, & prefrontal cognitive function) (Higley, Suomi, & Linnoila, 1992, 1996; Kaufman, Plotsky, Nemeroff, & Charney, 2000; McCormack, Sanchez, Bardi, & Maestripieri, 2006; Sanchez et al., 2001). These pathways are sensitive to HPA axis molecules, including CRF and cortisol. Sanchez (2006) suggests that attenuated ACTH responses to CRH injection indicate a potential down-regulation of CRH receptors in the pituitary following abnormally heightened CRH release due to chronic stress exposure (Sanchez et. al, 2010). The down-regulation of CRH can affect neuromodulatory regulation of the prefrontal cortex (PFC), and is implicated in reduced cognitive function (Lapiz et al., 2003).

Additionally, cortisol, a critical stress hormone, can cross the blood-brain barrier and bind to GRs and MRs that translocate to cell nuclei, ultimately affecting gene transcription of proteins involved in neurodevelopmental processes, including axon myelination (e.g., Chan, Phillips, & Glaser), as well as enhance the activity of proinflammatory cytokines in response to stress (Dhabhar, 2002). Proinflammatory cytokines can provoke cellular excitotoxicity (e.g.,Yuen, Wei, Liu, Zhing, Li, & Yan, 2012), as well as affect synthesis, turnover, and receptor binding of a number of different neurochemicals, including 5-HT, glutamate, GABA, and glucocorticoids. Sanchez et al. (2007) found that levels of proinflammatory cytokines in serotonin systems were higher in maltreated infant rhesus monkeys. Taken together, evidence

from these studies show that early life stress may have a number of different neurobiological mechanisms that can alter cognition, including disruption of axon myelination, cellular excitotoxicity, and altered neuromodulatory regulation of PFC-associated cognitive function.

The prefrontal cortex is comprised of a number of neuroanatomical and functional regions, among them the orbitofrontal PFC (ofPFC), which is primarily activated during tasks that require behavioral inhibition, such as reversal learning (e.g., Schoenebaum, Roesch, Stalnaker, & Takahashi, 2009), and the dorsolateral (dlPFC) and ventrolateral prefrontal cortex (vlPFC), key pathways involved in successful performance on working memory tasks (Barbey, Koenigs, & Grafman, 2011). One cognitive task that looks at actions of the ofPFC is the object retrieval detour (ORD) task, which uses a Plexiglas box with a single open side that allows the animals to reach in and retrieve the reward (see Diamond, 1990). The box opening can be turned away from the subject, so animals must be flexible in their strategy to solve the task, as well as exhibit inhibitory control over prepotent responses. Pryce and colleagues found that infant marmosets deprived of normal parental contact with HPA axis impairments similar to those found in rhesus monkeys (Sanchez 2006) committed more incorrectly perseverative reaches than controls on the ORD task (Pryce, Dettling, Spengler, Spaete, and Feldon, 2004). Working memory, on the other hand, is regulated by the vlPFC; the Delayed-Non-Matching-to-Sample Session Unique (DNMS-SU) task, for example, assesses working memory function through activation of the vlPFC (Mishkin & Delacour; Rapp & Amaral, 1989). DNMS tasks typically require the subject to choose the novel item (i.e., does not match) from a pair of objects following exposure to a model object. The Session Unique paradigm uses the same pair of objects across all trials, requiring the subject to shift from relying on recognition memory to solve the task to using working memory, since both items will appear as the model and the novel

object throughout the testing session. Nonhuman primate research using the ORD (Pryce et al., 2004) and the DNMS-SU (Heuer & Bachevalier, 2011a) to evaluate cognitive function suggest that compromised integrity of the PFC will elicit problems in inhibitory control, behavioral flexibility, and working memory.

There also is evidence that these pathways exhibit sexually dimorphic developmental patterns potentially mediated by androgen receptor binding (e.g., Clark & Goldman-Rakic, 1989). Clark and Goldman-Rakic found that male juvenile monkeys outperformed females on reversal learning tasks, which the authors attributed to the quicker development of the ofPFC in males. Humans also express sexually differentiated cognitive performance behavior, with females ages 8 – 21 outperforming males on attention and word and face memory, and males outperforming females on spatial reasoning and motor speed (Gur, et al., 2012). Inhibition and working memory were the two executive function behavior of interest in the present study's investigation of neurobiological factors involved in early-life stress related cognitive deficits, and sex differences in these domains were explored.

Rhesus Monkey Model

The current study used rhesus macaque monkeys (*Macaca mulatta)* as an animal model to investigate the impact of early-life stress on the development of CNS factors regulating cognition. The rhesus macaque model is the most frequently used comparative paradigm for exploring neurobiological consequences of early-life stress (Sanchez, 2006). Rhesus monkeys are excellent candidates for examining maternal maltreatment as a major early life stressor: They are highly social nonhuman primates with a prolonged mother-infant relationship and have analogous stress physiology to humans (e.g., Stevens, Leckman, Coplan, & Suomi, 2009). Furthermore, maltreatment occurs in 2-5% (Maestripieri, 1998) of captive, group-living

populations, similar to rates in human populations (Brent et al., 2002; Johnson, Kamilaris, Calogero, Gold, & Chousos, 1996; Maestripieri, 1999, 1998; Maestripieri & Carroll, 1998a, b; Maestripieri, Wallen, & Carroll, 1997a, b; Sanchez, 2006; Sanchez & Pollak, 2009; Sanchez et al., 2010; Troisi & D'Amato, 1984), and has been observed in wild macaque monkeys (e.g., Troisi, D'Amato, Fuccillo, & Scucchi, 1982).

Maltreatment in rhesus monkeys includes, abuse, rejection, and global dimensions of maternal care, including *sensitivity/responsivity*, *protectiveness*, *irritability*, and *attachment*. Maternal abuse and infant rejection are highly comorbid, and are distinct from the slaps or rejection behavior involved during weaning (Maestripieri, 1998). Abuse and rejection do not occur in nonabusive (control) mother-infant relationships. Abuse is operationally defined as the mother dragging, crushing, throwing, stepping or sitting on, or carrying the infant roughly; infant rejection is defined as preventing the infant from making nipple contact, either by holding the infant at a distance with an arm, passively blocking her chest with an arm, or by twisting her torso away from the infant (Maestripieri, 1998; McCormack et al., 2006). Maternal maltreatment triggers behavioral signs of distress (e.g., screams, tantrums) and HPA axis activation in infant macaques (Sanchez, 2006), signs that maltreated infants are experiencing high levels of stress.

Global dimensions of maternal care also are important for understanding the impact of maltreatment on infant and juvenile monkeys' behavior, emotionality, cognition, and neurobiology. Recent research addressing differences between maltreating and control mothers has shown that these maternal care dimensions, particularly sensitivity/responsivity, protectiveness, irritability, and attachment, are significantly altered in maltreating mothers (McCormack et al., personal communication). Furthermore, they seem to be better predictors of

behavioral (Ainsworth, Blehar, Waters, Wall, 1978; Atkinson et al., 2000; Paavola, et al., 2006) and neurodevelopmental (Howell, personal communication) outcomes than abuse or rejection alone. As in humans, maternal maltreatment in rhesus monkeys is associated with maladaptive behavioral, socioemotional, and neurobiological consequences.

Serotonin Transporter Gene Polymorphisms

Historically, allelic variations in the encoding gene for the serotonin transporter length promoter region (5-HTTLPR) have been examined in both humans and rhesus monkeys as an important genetic factor affecting emotionality and the stress response, as well as modulating vulnerability to early adverse care (e.g., Barr et al., 2003; Bennett et al., 2002, Champoux et al., 2002, Lesch et al., 2006). The gene encoding the 5-HT transporter, a molecule responsible for serotonin turnover through uptake into the presynaptic terminal, has two allelic variants, short (*s*) and long (*l*). The *s* allele is associated with reduced efficiency of 5-HTT transcription in human and nonhuman primates, leading to lower serotonin reuptake, a potential explanation for why *l*-variant monkeys tend to be more stress-resilient than their *s*-variant counterparts (Suomi, 2006). There also is some compelling evidence for a functional effect of length polymorphism on cognitive performance in both humans and rhesus monkeys (O'Hara et al., 2007). O'Hara et al. (2007) reported that in older adults (ages 60-100 years), the *s* allele was correlated with poor performance on the Rey Auditory Verbal Learning Test, a measure of delayed recall from verbal memory; individuals homozygous for the *s* allele (*s*/*s*) exhibited the poorest verbal memory. More current research, however, has reported that younger adults (ages 18-35 years) carrying the *s* allele performed better on a visual working memory task than individuals with *l*-variants (Anderson, Bell, & Awh, 2012).

Though the effect of polymorphisms of 5-HTTLPR on cognitive performance in humans is still not well understood, research using rhesus monkeys has shown clear evidence for the effect of allelic profile on cognitive function. On an object discrimination reversal learning (ODRL) task, *s/s* monkeys make significantly more errors before reaching criterion following the first reversal (Izquierdo, Newman, Higley, & Murray, 2007). In the first phase of the ODRL task, two objects were shown to the animals, one baited and one not baited, and the animal was able to displace the object and take the reward if the correct object was chosen. Criterion was met when the animal was accurate 93% of 30 trials one day followed by 80% accuracy the next. The next phase was the first reversal phase in which the original baited or "correct" object became unbaited and incorrect. Izquirdo et al. found that monkeys homozygous for the *s* allele made significantly more errors before reaching criterion on nine reversal trial phases, but not during the initial phase. Due to the evidence that the *l/l* profile for 5-HTTLPR may be an important protective factor in animals exposed to chronic stress and early adverse care, as well as decreased inhibitory control in cognitive performance shown by *s*-variant monkeys, the role of 5-HTTLPR on cognitive consequences associated with early adverse experience was addressed in the present study.

Current Study

The present study aimed to (1) examine the long-term effects of infant maltreatment on PFC-related task performance during the juvenile, prepubertal phase, and (2) address whether maltreatment-induced cognitive deficits are associated with neurochemical and neuroendocrine alterations in rhesus monkeys. To properly investigate how potentially altered (e.g., through stress-induced effects of elevated glucocorticoids on CNS, including altered myelination, proinflammatory cytokine activity, and cellular excitotoxicity discussed above) CRF and

monoamine neuromodulatory systems affect cognitive performance following early life stress, a number of factors were addressed: 1.) a variety of cognitive function domains, including inhibitory control, cognitive flexibility, perseveration, recognition memory, and working memory, 2.) subject sex, and 3.) vulnerability genetic factors, such as the 5-HTT *s* allelic variant described above. Evidence from previous research suggests that behavioral inhibition, impulse control, and working memory are particularly sensitive to chronic stress (e.g., Pears & Fisher, 2005; Pollak et al., 2010). The current study used cognitive tasks that examine PFC-related function, including a DNMS task to test working memory (Kowalska, Bachevalier, & Mishkin 1991; Malkova, Bachevalier, Webster, & Mishkin, 2000), and an object retrieval detour task (ORD) to test inhibitory control, reversal learning, and cognitive flexibility (Pryce et al., 2004).

The relationship between performance on cognitive tasks and rates of maternal abuse, rejection, restraint, and global dimensions of maternal care also was explored. Differences in maternal care dimensions, particularly sensitivity/responsivity, protectiveness, irritability, and attachment, between maltreated and competent (i.e., control) mothers are significant, with competent mothers displaying more behavioral patterns of responsivity to the infant, protection, and attachment, and maltreating mothers expressing an increase of irritability toward their infants (Bardi & Huffman, 2002; Bardi, Shimizu, Fujita, Borgoginni-Tarli, & Huffman, 2001; Maestripiri, Hoffman, Anderson, Carter, & Higley, 2009; McCormack et al., personal communication). Recent research addressing differences between maltreating and control mothers has shown that these maternal care dimensions were significantly altered in maltreating mothers (McCormack et al., personal communication). It was critical that we examined these global dimensions of maternal maltreatment in order to explore the qualities of maternal care that may have more impact on the behavioral (Ainsworth et al., 1978; Atkinson et al., 2000; Paavola,

et al., 2006) and neurodevelopmental (Howell, personal communication) outcomes of maltreated monkeys, rather than simply using frequency rates of abuse and/or rejection on their own.

Group-living rhesus monkeys were tested as juveniles, between 18-24 months of age, a critical developmental period for neural, social, and cognitive development (Heim & Binder, 2012; Suomi, 2005). A cross-fostering design, with random assignment of the infants at birth to either maltreating or control mothers, was used to control for potential effects of heritable and prenatal factors, including genetic influences on stress vulnerability/reactivity, cognitive performance, and neurobiological development. Cerebrospinal fluid (CSF) samples also were collected to examine the effects of maternal maltreatment on monoamine neurotransmitter and neuropeptide systems critical for stress and emotional regulation, impulse control, and cognitive function. For this, CSF concentrations of CRF and the monoamine metabolites: 5-hydroxyindoleacetic acid (5-HIAA) - the main 5HT metabolite-, 3-methoxy-4-hydroxyphenylgycol3 (MHPG), a norepinephrine metabolite, and homovanillic acid (HVA), a dopamine metabolite, were measured. All animals were genotyped for 5-HTTLPR polymorphisms to examine the potential effects of genetic variants in 5-HT function on cognitive performance.

Finally, maternal abuse, neglect, restraint, and maternal ratings collected with an instrument designed to measure global dimensions of maternal care (Appendix C, developed and used for previous Sanchez lab studies, McCormack et al., personal communication) were measured in maltreating and control mothers to examine whether maternal care during infancy (both positive maternal behavior – sensitivity/responsivity, protectiveness – and negative – abuse, rejection, irritability) was associated with performance on the cognitive tasks.

The predictions of the current study were:

1.) Maltreated juveniles would perform less accurately and efficiently than control animals on PFC-specific cognitive tasks, such as those designed to test behavioral inhibition and perseveration/cognitive flexibility (ORD) perseveration, and working memory (DNMS-SU). There would be no differences between groups, however, on control tasks that are not so critically independent on the PFC, such as those that test recognition memory (DNMS-TU) and general visual discrimination (Object Discrimination). Sex was expected to interact with experimental group such that control females were expected to learn the working memory task (DNMS-SU) the fastest and commit the least number of perseverative errors on the inhibition task (ORD) than all other groups. The 5-HTT genotype also was expected to moderate cognitive performance such that maltreated monkeys with an *s/s* or *l/s* allelic profile would exhibit the most cognitive task errors on both the ORD and DNMS-SU. Although our sample size did not allow us to examine the interaction effects of the 5-HTT genotype, its potential effects were controlled for in the statistical models used (described below) in order to assess its potential effect on cognitive measures and the effects of early adverse experience.

2.) Based on previous research, it also was expected that maternally maltreated rhesus monkeys would have higher CSF concentrations of CRF and lower CSF concentrations of 5-HIAA, HVA, and MHPG. Sex was expected to interact with experimental group such that control males were expected to have the highest concentrations of monoamine metabolites and lowest concentration of CRF (Higley et al., 1992)

3.) It was expected that 5-HIAA CSF levels would be inversely correlated with perseverative errors, partially explaining the effects of early life stress on impaired PFC-cognitive function expected above.

4.) It was predicted that cognitive function would be associated with the quality of maternal care received during infancy, such that *high* ratings on abuse, rejection, and irritability, and *low* ratings on sensitivity/responsivity, protectiveness, and attachment would correlate with poor performance on the ORD and DNMS-SU.

CHAPTER 2

METHOD

Subjects

Subjects were juvenile rhesus macaques (n = 36) already part of a longitudinal developmental study of effects of early maternal care, particularly infant maltreatment. Ten subjects (7 male, 3 female) were biological infants of control mothers and cross-fostered to maltreating mothers (control-to-maltreatment cross-fostered group), 7 subjects (2 male, 5 female) were in the maltreatment-to-control group, 12 subjects (6 male, 6 female) in the control-to-control group, and 8 subjects (5 male, 3 female) in the maltreatment-to-maltreatment group. Subjects were born between April and June across three birth years, 2009-2011.

Animals were socially housed 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. Subjects remained socially housed during the studies, which occurred from birth to the juvenile period. 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 items were provided once per day. All cognitive testing and sample collections were conducted after subjects reached 18 months of age. All procedures 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.

Procedure

Cross-Fostering and Measures of Maternal Behavior. Cross-fostering was used in order to rule out the potential confound of genetic and prenatal factors on developmental outcomes. Prior to this study and within the first two days of life, each subject was randomly assigned to be reared either by a control or maltreating multiparous foster mother, following a cross-fostering design to control for potential effects of "Biological Mother". These females were previously identified as either maltreating or non-maltreating (controls) based on documented maternal behavior in prior birth seasons. Their maternal behavior was confirmed following cross-fostering via focal observations of the infant-mother pair for 30 minutes, five times/week during the first month of life, two times/week during the second month of life, and one time/week during the third month of life. Maternal maltreatment in this model included both physical abuse and infant rejection. Maternal abuse was operationally defined as three or more occurrences of violent, infant-directed behavior. Abusive behavior included (1) Crushing the infant into the ground, (2) Dragging the infant along the ground, (3) Throwing the infant into the air, (4) Stepping on the infant, (5) Rough grooming the infant's hair too forcefully, and (6) Roughly carrying the infant so that the infant could not cling properly to her ventrum or back (see Table 1, Maestripieri, 2005; McCormack et al., 2006).

Maltreatment can cause significant infant distress, and extremely abusive females that seriously injure or kill their infants were excluded from the study. Maternal rejection was 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 the chest with an arm, or twisting her torso away from her infant (McCormack et al., 2006). Control mothers did not exhibit abusive behavior or rejection behavior that was atypical of weaning.

Table 1. Maternal Abuse Ethogram

Behavior	**Operational Definition**
Crushing	Pushes the infant against ground with hands
Dragging	Drags infant by tail while mother is walking or running
Throwing	Throws the infant ahead while mother is walking or running
Stepping on/Sitting on	Places feet or backside on infant and presses into the ground
Rough Groom	Holds infant on 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

Following each focal observation, a 22-item instrument designed to assess quality of global dimensions of maternal care, including maternal responsiveness, protectiveness, attachment, and irritability (Appendix C), was completed. This scale was developed from instruments used to evaluate the quality of mother-infant interactions in humans (Dolberg, Feldman, Keren, 2010; Gardner, Sonuga-Barke, & Sayal, 1999; Kok et al. 2012; Leerkes, 2011; Paavola, Kemppinen, Kumpulainen, Moilanen, & Ebeling, 2006; Pears & Ayres, 2000; Schneider-Rosen & Rothbaum, 1993; Vliegen, Luyten, & Biringen, 2009) and adjusted to evaluate rhesus macaque maternal behavior (Maestripieri, 1998, 2001; McCormack et al., 2006; McCormack et al., personal communication). Each item was scored on a 5-point Likert scale, with 1 representing no occurrence of the behavior (0%), 3 representing moderate occurrence of the behavior (26-50%) and a 5 representing a high occurrence of the behavior (76- 100%). If the

particular situation described in an item did not occur during the observation, then the event was scored as “N/A”.

Rhesus macaques are seasonal breeders, with breeding occurring in the fall months and births occurring in the spring, typically March through May at the YNPRC. Prior to the birth season, a list of potential foster mothers (i.e., multiparous females) was identified using YNPRC and laboratory records. 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 assessing fetal health. Cross-fostering was done within 48 hours of birth with multiparous females living in different social groups and mother-infant separations of 5 minutes or less (Maestripieri, 2005; Maestripieri, Jovanovic, & Gouzoules, 2000). The short separation time between mothers and infants, as well as the use of different social groups, are critical for successful cross-fostering (Guzman et al., 2012; Howell et al., 2012; Howell et al., 2011; Sanchez et al., 2011; Sanchez et al., 2012).

Animal Training. Prior to this study, all subjects were trained to move on command from the outdoor enclosure into an indoor capture unit using a point-and-clicker technique. The point-and-clicker technique required research staff to enter the outdoor corral of the home compound and point at the animals with a stick, simultaneously clicking a training clicker and guiding the animals into the indoor capture unit. Once the animals were inside the capture unit, they were trained to enter a transfer box and were habituated to the procedures for capture and CSF sampling. For the current study, animals were either transferred from the transfer box to a squeeze cage to be anesthetized for CSF collection, or remained in the transfer box to be transported to the Cognitive Testing room.

Cognitive Testing. Two cognitive tasks were used to assess the following PFC-related cognitive functions: 1.) The ORD task, which requires inhibitory control, reversal learning, and cognitive flexibility, and 2.) The DNMS Session Unique task (DNMS-SU), which tests working memory (testing on the DNMS-SU required previous acquisition of the rule of performance using a simple DNMS trial unique, or DNMS-TU). In addition, a visual recognition task (Object Discrimination task) was used to rule out alterations in visual function, basic memory processes, and/or general issues in motivation, attention, and reward. Cognitive testing began following all CSF and blood sample collection. On habituation, shaping, and testing days, animals were removed from their social group and transported to the Cognitive Testing room, located within a 5-minute cart ride from their home compound. There were two white-noise machines set up inside the room to muffle any outside disturbances. During the habituation and shaping phases for ORD and the habituation phase for DNMS-TU, personal comments (including food preferences, anxious behavior, and aggressive behavior), as well as latency and days to complete the phases, were recorded so the researcher could both qualitatively and quantitatively assess subject response motivation. Food deprivation was not used in these experiments; however, animals who displayed problems responding (i.e., did not participate in the task) following the habituation phase were placed in an adjacent room and were made to wait between 1-3 hours to increase motivation to complete the task by delaying their feeding schedule.

Object Retrieval Detour Task. In the ORD task (Jentsch, Roth, & Taylor, 2000), a Plexiglas box (10 cm x 10 cm x 10 cm) with one open side was attached to a testing platform by a moveable screw within a Wisconsin General Testing Apparatus (WGTA). Two tripods were placed across from the WGTA, one with a camcorder attached and the other with a security camera attached. The security camera allowed the experimenters to view the monkeys' behavior

from outside of the monkeys' visual range, as our physical presence caused anxious behavior. We first habituated and shaped animals to the task by placing food rewards on and around the transparent box. Food rewards consisted of small pieces of fruit (bananas, grapes), candy (i.e., Skittles, M&Ms), and popcorn. Food preferences were noted and reward choices were modified if necessary. The door of the WGTA was raised and the monkey was given 3 minutes to retrieve all of the food rewards. After all the rewards were retrieved, or after a maximum of 3 minutes if no response occurred, the door was lowered. The procedure was repeated until the animal reliably took rewards with little or no hesitation.

After the shaping phase was completed, the monkey began the ORD task. The ORD task was comprised of 7 testing days; Day 1 = 15 trials, Days 2-4 = 18 trials, and Days 5-7 = 21 trials. On Day 1 trials, a reward was placed on the edge of the open side of the box, facing the monkey, and only the box location changed, to either center, left of monkey, or right of monkey. On Days 2-4, box location, box opening orientation, and reward location within the box changed. In these trials, box opening orientation was rotated either 90^{o} or 180^{o}, with the box opening facing toward the monkey, to the right of the monkey, or to the left of the monkey. Reward location was placed at the box entrance, 1/4 of the way inside, 1/2 of the way inside, or 3/4 of the way inside. On Days 5-7, several trials in a row had the same box opening orientation and reward location, with box location consistently remaining in the center of the platform. After 6-7 trials in a row, the orientation of the box opening changed by 180^{o}. Reach difficulty was categorized based on box opening orientation and box location: 'Easy' trials were all trials in which the box opening faced the monkey, 'Moderate' trials were those in which the box moved from the center position to either lateral position, and 'Hard' trials were all trials in which the box was in the center position but changed 90^{o} from facing the monkey, or 180^{o} from facing one side of the testing

apparatus to the other. All trials across the seven days of testing were recorded for future coding for frequency of Balks (when the animal either did not attempt to retrieve the reward or made an initial attempt but then stopped making reaches before the end of trial time, 3 minutes), Reach Type (see Table 2), as well as Latencies to retrieve reward. All coding of videorecorded sessions were made using the Observer XT program (version 10.5, Noldus Information Technology, The Netherlands).

Table 2. Balk Characteristics and Reach Type on Object Route Detour (ORD) Task

Balks	**Operational Definition**
Balk with No Attempt	Monkey does not attempt to reach for the reward during a trial
Balk with Attempt	Monkey makes at least 1 reach during the trial, but stops making reaches before the trial time is over and does not retrieve reward
Reach Type	**Operational Definition**
Reach	Hand passes the entrance of the box opening; hand touches box corner
Barrier	Hand touches closed side of box
Global Perseverative	Consecutive barrier reach on same side as prior barrier reach
Reversal Perseverative	Barrier reach on side that was previously correct after the opening has been changed 180^{o} (Days 5-7 only)

Delayed-Non-Matching-to-Sample Session Unique (DNMS-SU). Following completion of the ORD task, animals were habituated to the DNMS testing platform and shaped to the paradigm. Meeting criterion on the DNMS Trial Unique task (DNMS-TU), which tests for recognition memory, was required before moving to the DNMS-SU, which assesses working memory. The testing tray for this task contained three recessed food wells spaced 10 cm apart, 5

cm in diameter, and 20 cm from the edge of the platform. During the shaping sessions, rewards initially were placed in all three of the wells at once and then gradually shifted into a single well at a time until the animal consistently retrieved the food items from each well. Since the DNMS-TU task also required animals to displace unfamiliar objects, the shaping procedure included the introduction of four different objects that were not used for future testing. The first object was placed near a baited well. When the monkey consistently retrieved the reward, the experimenter began to successively cover larger areas of the well until the monkey reliably displaced the object when it covered the entire well and retrieved the baited treat. The other objects were introduced to ensure that the animal would displace novel objects. DNMS-TU testing began when the animal consistently displaced all objects and retrieved the rewards.

The testing portion of the DNMS-TU task began immediately following shaping and included a 'familiarization' phase and a 'choice' phase. During the familiarization phase, a novel object called the *model object* was placed over the middle well containing a food reward. The screen was raised, the monkey was allowed to retrieve the reward, and then the screen was lowered. There was a 10-second delay between the familiarization and choice phase. In the choice phase, the model object was placed on either the left or right lateral well (predetermined by a pseudorandom order), with the novel object placed on the opposite lateral well. The reward was placed under the novel object. In order to retrieve the reward and correctly complete the trial, the subject had to displace the novel object. If the monkey displaced the model object, the door immediately was lowered and the next trial was presented following a 30-second interval (see Figure 1). The monkey was given 30 trials of DNMS-TU each testing day. The objects included pairs of at least 360 different unfamiliar items, varying in size, shape, color, and texture. In the DNMS-TU task, two different objects were presented per trial, for 30 trials per day.

Criterion was reached for the DNMS-TU task when the novel object was chosen 90 out of 100 consecutive trials.

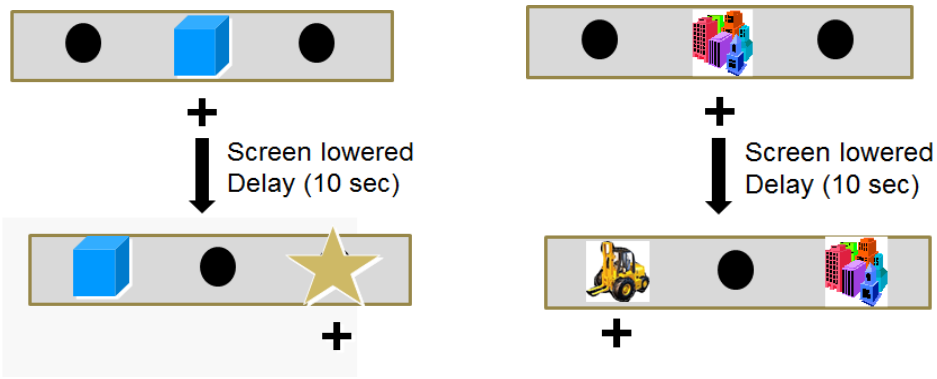

Figure 1. DNMS Trial Unique Testing Procedure for Two Consecutive Trials

After successfully reaching criterion for DNMS-TU, monkeys moved on to the DNMS-SU (Heuer & Bachevalier, 2011b). DNMS-SU is similar to DNMS-TU, but the same pair of objects is used across all trials in each session and only changes across days (testing sessions). During the familiarization phase for the DNMS-SU, one object of that pair was placed over the center well and acted as the model object. Following the monkey displacing the object and retrieving the reward, the screen was lowered and there was a 10-second delay. During the choice phase, the model object was placed on either of the left or right lateral wells and the novel object was placed on the opposite lateral well. Unlike the objects used in DNMS-TU, the same pair of objects was used throughout all trials in each testing session (day) for DNMS-SU (see Figure 2). Thus, starting with the second trial and for all remaining session trials, both objects were seen and baited such that successful performance required the animal to remember the object seen during the sample phase of a given trial (i.e., the object he had seen most recently).

The following day a new pair of objects was selected for the 30 daily trials and so on for all subsequent testing days. Learning criterion was set at 27 correct choices (90%) or better one day followed the next day by 24 correct choices (80%) or better. Testing was discontinued after a maximum of 1000 trials.

Figure 2. DNMS Session Unique Testing Procedure for Two Consecutive Trials

Object Discrimination. The final cognitive task was a simple Object Discrimination task designed to rule out the effect of group differences in visual recognition, motivation, attention, and/or reward. Two objects were placed over the lateral wells, which were both baited (Fernandez-Ruiz, Wang, Aigner, & Mishkin, 2001). Whichever object the monkey chose first continued to be the correct object choice. On the remaining trials, the two objects were placed simultaneously and pseudorandomly on the lateral wells. Criterion was reached when the monkey correctly selected the rewarded object 27 out of 30 trials for two days in a row.

CSF Sample Collection and Assays. CSF samples were obtained from all animals that participated in the cognitive studies. All subjects had prior CSF samples collected at 6 and 12 months of age as part of previous studies. The CSF samples were collected at sunrise to control for diurnal variations in neurochemical measures, and within 15 minutes of initial disturbance (i.e., researchers' arrival to the compound) to avoid stress-induced elevations. Subjects were

captured utilizing the training procedures described above and anesthetized with telazol (6mg/kg body weight, i.m.) immediately following transfer to a squeeze cage. CSF samples (0.5-1ml sample, collected as 1-2 aliquots of 500µl each) were collected via gravity from the *cisterna magna* with a sterile, 25-gauge needle following published protocols (Maestripieri et al.; 2006; McCormack et al., 2006; Sanchez et al., 2007). CSF samples were immediately stored in dry ice and then kept at -80^{o}C until assayed. To determine CRF concentration, samples were analyzed with a peptide enzyme immunoassay (EIA) from Peninsula Labs, LLC (San Carlos, CA) using an antiserum specific for CRF and a biotinylated – CRF to compete for binding sites on the antibody with the CRF in the CSF. All inter- and intra-assay variation was expected to be less than 10%. Additionally, to determine concentrations of 5-HIAA, HVA, and MHPG, the samples were analyzed using high performance liquid chromatography (HPLC) with electrochemical detection (HPLC-EC).

5-HTT Genotyping. Prior to this study, all monkeys were genotyped for 5-HTTLPR polymorphisms (see Hoffman, Kaplan, Kinkead, Berga, & Wilson, 2007). A 1.5-4.0 mL blood sample was taken and DNA extracted from whole blood using the Pure Gene Blood Kit (Gentra, D-4000). Polymorphisms in the promoter region of the 5-HT transporter gene were identified following amplification of relevant gene segments by polymerase chain reaction (PCR) using the oligonucleotide primers fwd (cag ggg aga tcc tgg gag gga) and rev (ggc gtt gcc gct ctg aat gc). The *s* amplicon (398 bps) and the *l* amplicon (419 bps) were separated on an agarose gel containing ethidium bromide and identified by direct visualization.

Data Analysis

Data was checked for normality and homogeneity of variance prior to statistical analyses. Non-normally distributed data was transformed using a square root transformation. When

transformed measures violated assumptions of normality and/or homogeneity of variance, nonparametric Mann-Whitney U analyses comparing control to maltreated subjects were conducted.

Group Differences. In order to explore the effect of maternal care (foster group assignment) and sex on ORD characteristics: i.) reaches, ii.) balks and iii.) latencies, while controlling for 5-HTTLPR polymporphisms, two-way ANCOVA analyses were conducted with sex and foster group assignment as fixed factors and 5-HTTLPR profile as a covariate. Reaches and balks were scored as frequency counts. We also conducted analyses on Day 2 of the ORD task; due to the challenge of Day 2, in which the animals first encountered changes in box orientation, they were more likely to balk than on Day 1 (in which no box orientation changes were made) or Day 4 (the final day of pseudorandom box orientation changes). A Chi-Square analysis was used to assess group differences on categorical data, balked versus did not balk, on Day 2 trials.

The effect of sex and foster group assignment on the DNMS-TU, DNMS-SU, and Object Discrimination performance characteristics: i.) Number of trials to criterion and ii.) Number of errors to criterion while controlling for 5-HTTLPR polymporphisms was explored using two-way ANCOVA analyses. Sex and foster group assignment were the fixed factors and 5-HTTLPR profile was included as a covariate. If animals showed consistent patterns of not responding to the task, or exhibited anxiety-related behavior such as stereotypies (i.e., rocking, circling, pacing), they were discontinued on these portions of the study. Thus, 13 animals failed to complete the DNMS-TU, 13 animals failed to complete the DNMS-SU, and 16 animals failed to complete the Object Discrimination task.

A two-way ANOVA was used to explore difference in CSF measures between maltreated and control animals, and the effects of sex. 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 (Barnett & Lewis, 1994). It was important to both include and remove outliers when examining the data: 1.) In the case for inclusion, an extreme data point was more likely to be drawn from either tail of the distribution curve due to the small sample size, and may have been a legitimately sampled score that was an outlier through random chance; 2.) In the case for exclusion, our sample size was not large enough to accommodate the possibility that there was standardization failure (i.e., on the day an animal was sampled, there was disturbance from construction, weather, etc.) or inadvertent sampling error.

Correlations. Pearson's Product Moment correlations were used to explore the relationship between maternal behavior (abuse, rejection, restraint, maternal care dimensions: sensitivity/responsivity, protectiveness, attachment, and irritability) with i.) reaches, ii.) balks, and iii.) latencies on the ORD task. Pearson's correlations also were used to investigate the relationship between ORD performances and the maternal behavior measures: i.) rates of abuse, ii.) rates of neglect, iii.) restraint, and iv.) scores on maternal rating scale: sensitivity/responsivity, protectiveness, attachment, and irritability.

Pearson's correlations were used to examine the relationship between CSF data and cognitive performance on the DNMS-TU, DNMS-SU, and Object Discrimination tasks. Pearson's correlations also were used to examine the relationship between measures of maternal care i.) rates of abuse, ii.) rates of neglect, and iii.) restraint, and iv.) scores on maternal rating scale and performance on the DNMS-TU, DNMS-SU, and Object Discrimination tasks.

Alpha levels were set at .05. All analyses were conducted using SPSS, version 21.0.

CHAPTER 3

RESULTS

Group and Sex Differences on Cognitive Tasks

ORD. Two-way ANCOVAs were conducted with foster group assignment and sex as fixed factors, performance characteristics on the ORD as dependent variables, and the functional polymorphism in the serotonin transporter gene (5-HTTLPR) as a covariate. There were no main effects of group assignment or sex, and there were no interaction effects on Total Reaches, Global Perseverative Reaches, or Reversal Perseverative Reaches, $p > .05$. There also were no main effects of group assignment or sex, and no interaction effects on Balks with Attempt, Balks with No Attempt, or Total Balks, $p > .05$. Finally, there were no main effects of experimental group assignment or sex, and no interaction effects on Day 1 Latency, Easy Trial Latency, Moderate Trial Latency, or Hard Trial Latency, $p > .05$.

DNMS-TU & DNMS-SU. Two-way ANCOVAs also were conducted with foster group assignment and sex as fixed factors, performance characteristics on the DNMS-TU, and the functional polymorphism in the serotonin transporter gene (5-HTTLPR) as a covariate. There were no main effects of group assignment or sex, and there were no interaction effects, on Trials to Criterion or Errors to Criterion for either DNMS-TU or DNMS-SU, $p > .05$

Object Discrimination. Two-way ANCOVAs also were conducted with foster group assignment and sex as fixed factors, performance characteristics on the object discrimination task as independent variable, and the functional polymorphism in the serotonin transporter gene

(5HTTLPR) as a covariate. There were no main effects of group assignment or sex, and there were no interaction effects on Trials to Criterion or Errors to Criterion, $p > .05$.

ORD Day 2 Analysis. The nature of Day 2 as the first challenging day of box orientation challenge also was examined in the groups. A Chi-Square analysis was used to explore differences between the groups on whether they attempted all trials, or did not attempt all trials; there were no differences between control animals and maltreated animals, $\chi^2 = 0.11$, $p = .74$. Some animals did attempt most trials, however, so another Chi-Square analysis was conducted, with criterion being attempted 15 (out of 18) or more trials; there was no difference between groups, $\chi^2 = 1.08$, $p = .30$. Males and females also were compared across Day 2 Motivation: No differences were found between males and females on Day 2 with strict boundaries (i.e., did not attempt at least 1 trial), $\chi^2 = 0.14$, $p = .71$, or with relaxed boundaries (i.e., did not attempt at least 3 trials), $\chi^2 = 0.30$, $p = .59$.

Group and Sex Differences in CSF levels of CRF and monoamine metabolites

Two-way ANOVA analyses indicated no significant group, sex, or group by sex effects for CSF concentrations of CRF, 5-HIAA, HVA, or MHPG, $p > .05$.

Correlations between Cognitive Task Performance & Maternal Care

ORD.

Although there were no correlations between maternal behavior and reaches, there was a positive correlation between Balks with Attempt to Retrieve Reward and Average rates of Rejection, $r = .362$, $p = .033$ (Table 3). Day 1 Latency was positively correlated with Restraint, $r = .409$, $p = .015$, and Responsivity, $r = .339$, $p = .339$, $p = .046$ (Table 4). All other correlations were not significant, $p > .05$.

DNMS-TU & DNMS-SU. Correlations were used to assess the relationship between performance characteristics of the DNMS-TU and DNMS-SU and maternal behavior, including rates of abuse, rates of rejection, and dimensions of Maternal Ratings scale (Appendix C). There were no significant correlations for DNMS-TU and maternal Behavior, $p > .05$. There were, however, positive correlations between DNMS-SU errors with Abuse, $r = .637$, $p = .003$, and Restraint, $r = .578$, $p = .008$ (Table 5).

Table 3. Correlations between Balks and Maternal Behavior

	Abuse	Rejection	Restraint	Responsivity	Protectiveness	Attachment	Irritability
Total Balks	.041	.066	.080	.141	.108	.032	-.006
Balk with Attempt	.261	**.362***	-.186	-.044	-.005	-.072	.331
Balk with No Attempt	-.082	-.095	.140	.181	.124	.108	-.097

* Correlation is significant at the .05 level (2-tailed).

Table 4. Correlations between Latencies to Retrieve Reward and Maternal Behavior

	Abuse	Rejection	Restraint	Responsivity	Protectiveness	Attachment	Irritability
Day 1 Latency	-.127	-.090	**.409***	**.339***	.303	.155	-.149
Easy Trial Latency	-.041	.050	-.108	.027	-.066	-.052	.059
Moderate Trial Latency	.118	.130	.150	.150	.212	-.074	.033
Hard Trial Latency	.174	.112	.200	.115	.171	-.153	-.034

* Correlation is significant at the .05 level (2-tailed).

Table 5. Correlations between DNMS-SU performance characteristics and Maternal Behavior

	Abuse	Rejection	Restraint	Responsivity	Protectiveness	Attachment	Irritability
DNMS-SU Trials	.278	.128	.257	-.042	-.026	-.039	.250
DNMS-SU Errors	**.637****	.256	**.578****	-.228	-.179	-.334	.350

** Correlation is significant at the .01 level (2-tailed).

Object Discrimination.

Correlations revealed a positive relationship between Object Discrimination errors and rates of rejection, $r = .633$, $p = .002$, as well as a positive relationship between trials to criterion and Abuse, $r = .607$, $p = .004$, and Restraint, $r = .462$, $p = .035$ (Table 6). All other correlations were nonsignificant, $p > .05$.

Table 6. Correlations between Object Discrimination variables and Maternal Behavior

	Abuse	Rejection	Restraint	Responsivity	Protectiveness	Attachment	Irritability
Object Discrimination Trials	**.607****	.277	**.462***	-.188	-.197	-.234	.281
Object Discrimination Errors	.341	**.633****	.113	-.376	-.420	-.364	.408

** Correlation is significant at the .01 level (2-tailed).

* Correlation is significant at the .05 level (2-tailed).

Correlations between Performance on Cognitive Tasks and CSF Concentrations of CRF & Monoamine Metabolites.

ORD.

Correlations between reaches and CSF concentrations revealed a negative correlation between the 5-HT metabolite, 5-HIAA, and Barrier reaches, $r = -.468$, $p = .006$, and Reversal Perseverative reaches, $r = -.367$, $p = .036$ (Table 7). All other correlations were nonsignificant, $p > .05$.

Table 7. Correlations between Reach Type and CSF concentrations of CRF and monoamine metabolites at 18 months

	CRF	MHPG	5-HIAA	HVA
Total	-.075	.019	-.200	-.136
Barrier	-.116	-.226	**-.468****	-.263
Global Perseverative	-.051	-.342	-.342	-.274
Reversal Perseverative	-.119	-.314	**-.367***	-.193

** Correlation is significant at the .01 level (2-tailed).

* Correlation is significant at the .05 level (2-tailed).

Correlations between Balks and CSF concentrations of CRF and monoamine metabolites revealed a positive correlation between Balks with No Attempt and CRF, a neuropeptide involved in the behavioral, neuroendocrine, and sympathetic stress response, $r = .374$, $p = .029$ (Table 8). Correlations between Latencies to retrieve the reward and CSF concentrations of CRF and monoamine metabolites revealed a positive correlation between CRF and Moderate, $r = .452$, $p = .007$, and Hard Trial latencies, $r = .475$, $p = .005$ (Table 9). The dopamine metabolite, HVA, was correlated with Day 1 Latency, $r = .605$, $p = .000$. All other correlations were nonsignificant, $p > .05$.

Table 8. Correlations between Balks and CSF Concentrations of CRF and monoamine metabolites at 18 months

	CRF	MHPG	5-HIAA	HVA
Total Balks	.321	.127	.258	.215
Balk with Attempt	-.087	.145	.169	-.063
Balk with No Attempt	**.374***	.066	.186	.248

* Correlation is significant at the .05 level (2-tailed).

Table 9. Correlations between Latencies and CSF Concentrations of CRF and monoamine metabolites at 18 months

	CRF	MHPG	5-HIAA	HVA
Day 1	.295	.327	.245	**.605****
Easy Trial	.222	.012	.022	.062
Moderate Trial	**.452****	.105	.234	.102
Hard Trial	**.475****	.229	.175	.210

** Correlation is significant at the .01 level (2-tailed).

DNMS-TU & DNMS-SU. Correlations were used to assess relationship between performance characteristics on the DNMS-TU and DNMS- SU with CRF and monoamine metabolites. All correlations were nonsignificant, $p > .05$.

Object Discrimination. Performance on the Object Discrimination Task was correlated with CSF concentrations. Errors to criterion were positively correlated with MHPG, $r = .542$, $p = .013$ (Table 10). All other correlations were nonsignificant, $p > .05$.

Table 10. Correlations between Object Discrimination performance characteristics and CSF Concentrations of CRF and monoamine metabolites

	CRF	MHPG	5-HIAA	HVA
Object Discrimination Trials	-.011	.302	-.047	-.068
Object Discrimination Errors	.271	**.542***	.304	.075

* Correlation is significant at the .05 level (2-tailed).

CHAPTER 4

DISCUSSION

Although no significant effects of group assignment were detected on either the Object Retrieval Detour (ORD) or Delayed-Non-Matching-to-Sample Session Unique (DNMS-SU) tasks, or in the CSF levels of the stress neuropeptide CRF or concentrations of monoamine metabolites (5-HIAA, HVA, MHPG) (Maestripieri et al., 2006; Vratanina-Smoot, personal communication), we found a number of strong significant correlations between performance variables on the cognitive tasks and maternal behavior, as well as between cognitive performance and CSF measures. These relationships provide us with deeper insight into how early life experiences, particularly variations in maternal care during the first three months of life, as well as neurobiological correlates of PFC-associated function, may affect cognition in juvenile rhesus monkeys.

Maternal Care and Cognitive Task Performance

On the ORD task, we found a correlation between rates of rejection received during the first three months of life and balks with an attempt to complete the task, such that animals who experienced higher rates of rejection were more likely to exhibit a decrease in motivation to complete the trial if they were initially unsuccessful. This relationship could potentially reflect learned helplessness in these animals. Seligman's theory of learned helplessness in the context of maternal attachment posits that when animals who are highly reliant on maternal care in early life have an uncertain relationship with their mothers, learning is impaired due to a history of ineffective goal-directed behavior (Seligman,1975; Mineka & Suomi, 1978). A potential

explanation for our findings is that these animals could exhibit lower motivation to respond, due to past failure to exert control over their environment, and subsequently perform poorly on cognitive tasks. Rather than persist in their pursuit of the goal, these animals stop responding. In the current study, the learned helplessness hypothesis may explain why animals rejected by their mothers stop reaching after initial, and unsuccessful, reach attempts on the ORD task.

We also found a positive correlation between ORD Day 1 Latency to retrieve the food reward with both maternal restraint and responsivity. Animals who experienced higher levels of restraint and responsivity by their mothers were more likely to take longer to retrieve the reward on the first day of ORD testing, indicating hesitance to approach the platform on each trial. Restraint and responsivity are both aspects of competent maternal patterns in that monkey mothers who hold and attend to their infants are exhibiting healthy maternal behavior, as compared with mothers who reject and neglect their infants' need for contact (e.g., McCormack et al., personal communication). It is possible that animals who were raised by highly responsive/sensitive mothers (and were therefore more likely to be securely attached) and monkeys raised by mothers who were more restraining (protective) may have been more hesitant to approach novel stimuli such as food rewards in the testing apparatus. While increased latencies can potentially be interpreted as reflecting more stress and/or anxiety during the first day of the task, they do not indicate the same lack of willingness to complete trials as animals who balk, therefore suggesting a different underlying behavioral state.

We also found a significant positive correlation between errors to criterion on the DNMS-SU task with rates of maternal abuse, suggesting maltreatment-associated impairment in working memory. Heuer and Bachevalier (2011a) found that lesions to the hippocampus, a brain structure involved in working memory with novel items (Ranganath & D'Esposito, 2001),

caused adult rhesus monkeys to make more errors with longer intertrial times on a working memory task as they aged. Subsequently, Bremner et al. (1997) reported that victims of sexual abuse had significantly lower hippocampal volume than controls, findings confirmed by other research groups (e.g., Stein, Koverola, Hanna, Torchia, & McClarty, 1997). Surprisingly, errors to criterion also were associated with restraint. Again, it is possible that this relationship may be similar to the increase in latencies on Day 1 of the ORD task seen in animals who experienced higher frequencies of restraint: While the animal was willing to complete the task, stress resulting from maternal separation may have compromised cognitive performance.

CSF Concentrations of Monoamine Metabolites & CRF and Cognitive Task Performance

The regulation of the PFC by 5-HT is explained by the strong serotonergic innervation of the PFC by the raphe nuclei (for a review, see Celada, Puig, & Artigas, 2013), thus supporting 5-HT's role in inhibitory control and cognitive flexibility (e.g., Garner, Wood, Pantelis, & van den Buuse, 2007; Robbins, Jones, & Wilkinson, 1996). Selective depletion of serotonin in the PFCs of marmosets revealed that monkeys receiving multiple injections of 5,7-DHT (a neurotoxin that selectively targets serotonergic cells) in the PFC made more perseverative errors than controls on reversal trials in a pre-operatively learned reward contingency test (Clarke, Dalley, Crofts, Robbins, & Roberts, 2004). Additionally, Murai and colleagues showed that lurasidone, an antipsychotic antagonist for D2, 5HT7, 5HT2 receptors, enhanced marmosets' performance on the Object Retrieval Detour task (Murai et al., 2013). Thus, the association between lower levels of 5-HIAA, the main 5-HT metabolite, and higher frequencies of barrier and perseverative reaches in our studies is consistent with the previous evidence supporting an important role of 5-HT on PFC inhibitory control of behavior and cognitive flexibility (Clarke et al., 2004; Izquierdo et al., 2007; Murai et al., 2013).

Success on the ORD task requires activation of the ofPFC. In the ORD task, perseverative reaches are a critical behavior associated with inhibitory control and cognitive flexibility, as subjects either must change their reach to avoid a clear barrier (Days 2-4), or inhibit a prepotent response (Days 5-7) (Iversen & Mishkin, 1970). It has been shown that ablations to the ofPFC, a neural substrate vulnerable to the effects of early adverse experiences, causes monkeys (e.g., Dias et al., 1996) and humans (Fellows & Farah, 2003) to perseverate more on reversal learning tasks. Neuroimaging studies in human populations with histories of childhood maltreatment show that this area is not activated when subjects are asked to perform tasks that require inhibitory control, with subjects making more perseverative mistakes and exhibiting a dampening of cognitive flexibility (Hart & Rubia, 2012). When taken together with the 5-HIAA correlation data, the present study indicates that serotonergic afferents to the ofPFC seem critical for inhibitory control and cognitive flexibility necessary to perform efficiently on the ORD task. Alterations in neuroendocrine function resulting from early life stress (e.g., elevations in cortisol) can compromise prefrontal cortex function (e.g., Howell et al., 2011), including inhibitory control and memory (e.g., Lyons, Lopez, Yang, & Schatzberg, 2000).

Other important behaviors that were examined on the ORD were *balks*, in which the animal either did not attempt any reach during a trial or made some reaches but stopped participating before the trial time was over, and *latencies to retrieve reward*. CRF, a critical neuropeptide involved in the stress response, was correlated with balks with no attempt, such that animals with high CSF concentrations of CRF were more likely to not make any reaches on ORD trials than animals with lower concentrations of CRF. CRF mediates the behavioral, neuroendocrine, autonomic, and cognitive responses to stress, so our findings are unsurprising and consistent with the literature (e.g., Kalin, Shelton, Kraemer, & McKinney, 1983; Munck,

Guyre, & Holbrook, 1984; Radulovic, Rühmann, Liepold, & Spiess, 1999). CRF receptors are distributed throughout the rhesus monkey PFC and the amygdala, which has strong connectivity with the PFC and is critical for emotional regulation (Sanchez, Young, Plotsky, & Insel, 1999), supporting a role of CRF in the regulation of PFC-mediated cognitive and affective function. Furthermore, acute restraint stress in rats has been shown to increase CRF mRNA expression in the PFC (Meng, Chen, Tong, & Zhou, 2011), and overexpression of forebrain CRF (mouse model) has been shown to impair spatial learning and memory (Wang et al., 2011). Though CRF was not associated with "making mistakes" on the ORD through increased barrier or perseverative reaches in the current study, the positive correlation between CRF and balks with no attempt indicates a dampening of executive function that may underlie cognitive deficits typically associated with elevated central levels of CRF.

Levels of HVA, a dopamine metabolite, were positively correlated with Day 1 Latency, indicating potential alteration of activity in striatocortical pathways involved in executive function (Rodrigues, Leãs, Carvalho, Almeida, & Sousa, 2011). Dopaminergic innervations projecting from the ventral tegmental area to the mesolimbic and mesocortical pathways are highly involved in regulating appetitive behavior (i.e., motivation; for a review, see Wise, 2004). Compromised dopaminergic tone in the mesolimbic and mesocortical pathways are associated with schizophrenia, depression, ADHD, behavioral impulsivity, and other pathologies comorbid with cognitive dysfunction seen in individuals with early adverse experience (Rodrigues et al., 2011). HVA correlation with increased latencies, in conjunction with the association between CRF and balks with no attempt and the relationship between maternal rejection and balks with attempt, indicate that motivation (a striatum-mediated function, Neill, 1976) may have been compromised in a number of our animals as a potential consequence of learned helplessness.

We did not find any correlations between CSF measures and performance on the DNMS-SU task. In the DNMS-SU, fewer trials to criterion and fewer errors to criterion reflected efficiency in using working memory to complete the task. It is established in nonhuman animal studies that lesions to the hippocampus affect performance on working memory tasks (e.g., François et al., 2009; Zeamer, Heuer, & Bachevalier, 2010), and that the prefrontal cortex mediates the role of the hippocampus on performance. Developmental studies show that early life stress can reduce hippocampal plasticity and enhance hippocampal vulnerability in rats (Jin et al., 2013), and may compromise dopaminergic tone in mesocortical pathways (Rodrigues et al., 2011). It is possible that because we began testing our animals at 18 months of age, we were unable to successfully assess some of the true PFC deficits seen in older animals with a history of early adverse experience. Our animals experienced early life stress through the entirety of infancy, and were tested during the juvenile period. In rhesus monkeys, synaptogenesis is prolific in the PFC from birth through approximately 2 months of age (Bourgeois, Goldman-Rakic, & Rakic, 1994), at which time synaptogenesis begins gradually to decrease and synaptic pruning and myelination increase. This myelination also rapidly and drastically increases until approximately 12 months of age, when the PFC continues to mature more gradually into adulthood. The neuroplasticity of the PFC during infancy correlates with the period in which maltreated animals experience the highest levels of abuse and neglect (McCormack et al. 2006); from measures taken from ages 3-18 months, Howell et al. (2011) found that white matter in the PFC of maltreated rhesus monkeys was significantly less developed compared with controls. It is possible that our animals may have been too young to account for the intense proliferation of synaptic pruning in the PFC that may delay early adverse impact on cognitive performance. It also is important to consider an alternative explanation, that because these animals completed the

ORD task before proceeding to the DNMS tasks, they were highly habituated to testing and therefore did not exhibit a strong behavioral or physiological response to the novel testing paradigm.

An alternative perspective to understanding our animals' performance is that cognitive processes have been shaped rather than impaired by poor maternal care, a broader definition than considering abuse as the sole maltreating factor (Frankenhuis & deWeerth, 2013). Abused children are quicker at detecting and processing angry faces and potentially aggressive social partners than their control counterparts, perhaps due to the ecological relevance this information holds. Subsequently, they do not perform as well on traditional psychometric assessments and testing paradigms, which may be more relevant to nonabused populations who can direct more cognitive resources to traditional cognitive performance evaluation measures. It is possible that because the cognitive tasks we used in the present study are not ecologically salient to either group, we were unable to elicit group differences that may reflect the differential development of neural processes necessary for our subjects to perform successfully on these cognitive tasks.

Another potentially valid predictor that was relatively unexplored in the present study is gene by environment interaction effects. Although we indirectly addressed a role of epigenetic factors in our model by controlling for 5-HTTLPR polymorphisms, there are a number of other factors involved in developmental changes in neural plasticity that have lifelong structural and functional effects in PFC pathways of interest (McEwen, 2007). In order to investigate McEwen (2007) and others' (e.g., Smith, Makino, Kvetnansky, & Post, 1995) hypothesis that cognitive deficits associated with early life stress manifest as a result of potentially aberrant changes in neural plasticity in associated areas such as the hippocampus, amygdala, and prefrontal cortex, recent research looking at the role of brain-derived neurotrophic factor (BDNF) in regulating

effects of early life stress may reveal individual differences in associated biological changes (e.g., Roth, Lubin, Funk, & Sweatt, 2009). BDNF is a neurotrophin that is critical for regulating neural integrity and pathway formation. In maternally abused and deprived animals, protein levels and mRNA expression of BDNF are downregulated in animals with exposure to early life stress. Using a rat model of childhood maltreatment with cross-fostering, Roth et al. found that maltreated animals had blunted mRNA expression of BDNF in the PFC, but not the hippocampus (Roth et al., 2009), and that these changes persisted through adulthood. Additionally, these patterns persisted in the next generation of rats, such that maltreated females gave birth to pups who also displayed these patterns. Roth et al. describe an interaction effect of postnatal treatment by the mother, as females who had been maltreated as pups were more likely to display abusive behavior to their own offspring; unlike with first generation animals, however, those who were cross-fostered did not recover down-regulation patterns of mRNA expression with methylation treatment, indicating the effect of epigenetic factors rather than simply exposure to maternal abuse.

While the work of Roth et al. (2009) may seem to contradict effects seen in the present study, since our animals also were cross-fostered, other research looking at the *epigenomic* effects may lend more insight. Etzinga and colleagues looked at how polymorphisms of the $Val^{66}Met$ gene, which codes for BDNF, may moderate the effects of the relationship between BDNF and consequences of childhood maltreatment (Etzinga, et al., 2011). Met carriers typically express less secretion of BDNF than Val carriers, and display consequences discussed by previous researchers in regard to down-regulation of BDNF (McEwen, 2007; Smith et al., 1995). In looking at both childhood abuse victims and nonabused individuals, all with a history of Major Depression, Etzinga et al. found that individuals with the Met allele showed reduced

serum levels of BDNF when exposed to child abuse. BDNF in Val/Val individuals was not affected by childhood abuse. In the context of the present study, we do not have information about the Val^{66}Met polymorphisms in our subjects, nor the serum levels of BDNF. The work from Roth et al. (2009) indicates the relationship among BDNF, early life stress, and neural plasticity may powerfully affect cognition in maltreated individuals; the work from Etzinga et al. (2011) also supports the importance of the genetic profile, as the Val allele may be the key to having neuroanatomical resiliency in regard to childhood maltreatment (Roth & Sweatt, 2011). While past epigenetic research primarily has focused on effect of 5-HTTLPR polymorphisms on the cognitive consequences associated with early adverse experience. While the present study looked at 5-HTTLPR polymorphisms, it is compelling to consider the effect of BDNF.

Conclusion

Evidence from the current study indicated that there is an important association between maternal behavior dimensions (sensitivity/responsivity, protectiveness, attachment, and irritability) and cognitive task performance, which may better predict cognitive function than rates of abuse and rejection alone. Furthermore, we found significant correlations between CSF concentrations of monoamine metabolites and CRF and cognitive function. Taken together, we show evidence for the effect of learned helplessness and stress-related decreases in motivation on cognitive task performance. Additionally, our animals displayed compromised performance on tasks designed to test function of the PFC, indicating that despite the lack of group effects, alteration of critical neuromodulatory systems can result in attenuation of inhibitory control, cognitive flexibility, and working memory. While we did not find evidence for interaction effects of 5-HTTLPR polymorphisms, it is critical to explore other epigenomic factors, such as

Val/Met polymorphisms for BDNF synthesis and turnover, that may reveal more insight into potential protective factors established at the genetic level.

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APPENDIX A

LITERATURE REVIEW

Childhood maltreatment includes both abuse and neglect, and can result in severe behavioral, emotional, and cognitive consequences (e.g., Beers & DeBellis, 2002; Carrey, Butter, Persinger, & Bialik, 1995). Abuse can be physical (i.e., hitting, kicking, burning; Sedlak & Broadhurst, 1996), sexual (any sexual activity with a child, including watching pornography or involving child in pornography; Garbarino & Garbarino, 1994), and/or emotional (i.e., verbal abuse, exposing child to domestic violence; Putnam, 2003). Neglect occurs when caretakers do not attend to the child's need for affection or emotional support, do not provide proper shelter or nutritional needs, allow regular school absences, and/or permit alcohol and drug use (English, Thompson, Graham, & Briggs, 2005).

Early adverse experience can have serious effects on socioemotional behavior. Victims of abuse are more likely to have negative social interactions with their peers (Haskett & Kistner, 1991; Jacobson & Straker, 1982), and display increased aggression and engage in less play than nonabused children (e.g., Allesandri, 1991). Abused children also are more likely to be antisocial, display conduct disorder, and be more disruptive than nonabused children (Hart, Gunnar, & Cicchetti, 1995; Fagot, Hagan, Youngblade, & Potter, 1989). Furthermore, child victims of abuse and neglect are predisposed to clinical pathologies and emotional regulation problems, including major depression and PTSD (Bremner, Southwick, Johnson, Yehuda, & Charney, 1993; Mullen, Martin, Anderson, Romans, & Herbison, 1996; Stein et al., 1996), as

well as heightened suicidality, anxiety disorders, and problems with addiction (DeBellis et al. 1994; Weiss, Longhurst, & Mazure, 1999).

Abuse and neglect also may alter normal cognitive development. Maltreatment is associated with lower IQ scores and poor academic performance (i.e., Majer, Naker, Lin, Capuron, & Reeves, 2010), language and memory impairments (Bos, Fox, Charles, & Nelson III, 2009), and inhibitory control problems (see Pechtel & Pizzagalli, 2010, for a review). Executive function deficits can emerge in victims of maltreatment at an early age and continue on into adulthood (Spann et al., 2012). These impairments often are specific to certain types of cognitive function (e.g., Pears & Fisher, 2005; Pollak et al., 2010). Pollak et al. (2010) found that while maltreated children (8 – 9 years of age) in their study performed as well as controls on some executive functions, such as rule acquisition and planning, they displayed working memory and behavioral inhibition problems. It is likely that distinct neural circuitry is differentially affected by these early adverse experiences, specifically pathways in the prefrontal cortex, or PFC (Lyons, Lopez, Yang, & Schatzberg, 2000). Mueller et al. (2010) showed that adopted adolescents who had experienced early life stress through parental maltreatment, neglect, or unstable family environments performed more slowly than controls on 'change strategy' trials on a stop-signal cognitive task, which assesses participants' ability to inhibit a prepotent response to a nonprepotent response. The increased latencies seen in the experimental group correlated with more activation in the inferior prefrontal cortex and striatum. Work with marmoset monkeys showed that distinct pathways control different aspects of cognitive functions: Monkeys with lesions to the orbitofrontal PFC (ofPFC) took significantly longer than animals with lesions to the lateral PFC and controls to reach criterion on a reversal learning; in contrast, lesions to the lateral PFC impaired monkeys' ability to successfully complete a set-shifting task, but not

lesions to the ofPFC (Dias, Robbins, & Roberts, 1996). Although there is evidence that there are cognitive consequences associated with maltreatment as a form of early life stress, how, when, and why these alterations develop through infancy and juvenile periods are questions researchers are still working to answer. This review will discuss some of the most relevant literature concerning the neurobiological substrates potentially associated with cognitive deficits seen in maltreated humans and nonhuman animals.

Neurobiology of Maltreatment

From a neurobiological perspective, maltreatment is considered a form of early life stress, sometimes extremely severe, that causes powerful activation of stress response systems. These systems include the sympathetic-adrenomedullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) axis, as well as monoaminergic neuromodulatory projections involved in the stress response (Gunnar & Herrara, 2007; Gunnar & Quevedo, 2007; Gutman & Nemeroff, 2003; Sanchez & Pollak, 2009). Both the HPA axis and the SAM system are regulated by the hypothalamus, a central nervous system (CNS) structure that governs endocrine, behavioral, and autonomic functions via a number of neural inputs, notably projections from the brain stem. These projections include inputs from the locus coeruleus (a pontine nucleus), serotonergic afferents from the raphe nuclei, and catecholaminergic projections from the nucleus of the solitary tract (Gunnar & Herrera, 2007; Ulrich-Lai & Herman, 2009). As part of the stress-induced activations of the sympathetic branch of the autonomic nervous system, the stimulation of the neuroendocrine SAM system results in both behavioral and physiological responses to stressors through release of norepinephrine and epinephrine by the adrenal medullas into the bloodstream, causing increased heart rate (part of the “fight or flight” response), peripheral vasoconstriction, and energy mobilization.

Simultaneously, CNS pathways are involved in preparing the organism to respond to the stressor by enhancing arousal, increasing vigilance, and assisting with activation of the HPA axis. The HPA axis is activated by the actions of the paraventricular nucleus (PVN) of the hypothalamus, which releases corticotropin-releasing factor (CRF) into the portal blood circulation at the same time the SAM is activated. CRF travels through this hypophyseal portal system, a web of capillaries connecting the hypothalamus to the pituitary gland, and binds to CRF receptors in the adenohypohysis (anterior pituitary gland). In response to CRF binding, the adenohypophysis secretes adrenocorticotrophin hormone (ACTH) into the bloodstream. ACTH binding in the adrenal cortex results in glucocorticoid (cortisol/corticosterone) synthesis and release, highly catabolic steroid hormones that ultimately bind to receptors in target tissues. The HPA axis is much slower in responding to stress than the SAM system, with glucocorticoids taking several minutes to be synthesized, released, transported and bound to their receptors. Glucocorticoids bind to intracellular mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), mediating their actions mainly through regulation of gene expression and also can cross the blood brain barrier, affecting CNS function.

In addition to the HPA axis and SAM system, behavioral, autonomic, and neuroendocrine stress effects and responses are regulated by certain monoaminergic neuromodulatory pathways, including the serotonergic system (5-HT; involved in emotion regulation and impulse control), noradrenergic projections (arousal, threat assessment), and dopaminergic pathways (appetitive behavior, behavioral inhibition, attention, and other prefrontal cognitive functions) (Higley, Suomi, & Linnoila, 1992, 1996; Kaufman, Plotsky, Nemeroff, & Charney, 2000; McCormack, Sanchez, Bardi, & Maestripieri, 2006; Sanchez, Ladd, & Plotsky, 2001). These pathways are sensitive to HPA axis activity, including CRF and cortisol actions. Chronic stress can result in

long-term behavioral, neuroendocrine, and neurobiological consequences (Sanchez, 2006), including increased anxiety and emotional reactivity (e.g., McCormack et al., 2006), hypo- and hypercortisolemia (e.g., Gillespie & Nemeroff, 2005), and blunted ACTH responses to CRH injection (Sanchez et al., 2010). The attenuated response to this biological stress challenge suggests a possible down-regulation of CRH receptors in the pituitary following abnormally heightened CRH release due to chronic stress exposure (Sanchez et. al, 2010). CRH down-regulation, combined with other mechanisms such as cellular excitotoxicity (e.g.,Yuen, Wei, Liu, Zhing, Li, & Yan, 2012), and reduced 5-HT tone (e.g., Lapiz et al., 2003), can impair prefrontal cortex (PFC) function.

The PFC is comprised of different neuroanatomical and functional regions, including the orbitofrontal PFC (ofPFC), ventrolateral PFC (vlPFC), and the dorsolateral PFC (dlPFC). The ofPFC is primarily activated during tasks that require behavioral inhibition, such as reversal learning (e.g., Schoenebaum, Roesch, Stalnaker, & Takahashi, 2009) and the vlPFC and dlPFC are critical for attention, successful performance on working memory tasks, and also top-down behavioral control (Barbey, Koenigs, & Grafman, 2011). Early life stress significantly affects prefrontal cortex development (Howell et al., 2011), and rhesus juveniles raised in a nursery without contact with their mothers exhibit reduced prefrontal cortex white matter volume (Sanchez et al., 1998).

The prefrontal cortex also is potentially affected by early life stress through stress-induced attenuated immune function in the CNS, which may occur as a necessary component of leukocytes migrating to more peripheral systems to prepare for potential injury (Dhabar, 2002). Following the movement of leukocytes, expression, entrance, and/or release of proinflammatory cytokines in the CNS may occur. In the brain, these cytokines may affect synthesis, turnover,

and receptor binding of a number of different neurochemicals, including 5-HT, glutamate, GABA, and glucocorticoids. Through measuring of monocytes, Sanchez et al. (2007) found that proinflammatory pathways in serotonin systems were greater in maltreated infants. The effects of stress, chronic stress, and early life stress on prefrontal cortex function are demonstrated through a number of different physiological mechanisms (e.g., Kern et al., 2008; Sanchez, 2006).

Rhesus Monkey Model

Early studies in Harry Harlow's lab (Harlow & Harlow, 1969; Harlow, Harlow, & Suomi, 1971) examined effects of isolation rearing on young rhesus macaque monkeys. Isolate-reared infants displayed a number of behavioral differences than monkeys raised by their mothers, including stereotypies (such as rocking, and pacing), self-clutching, blank-staring, and self mutilation. Furthermore, they exhibited poor social behavior when placed with control monkeys of the same age, failing to make contact or play with other animals. Female monkeys reared in isolation also were more likely to reject and abuse their own infants, one example of a developmental behavioral consequence of maternal deprivation (Seay, Alexander, & Harlow, 1964).

The rhesus macaque continues to be one of the most frequently used primate species for exploring neurobiological consequences of early life stress (Sanchez, 2006). Rhesus monkeys are excellent candidates for examining maternal maltreatment as a major early life stressor: They are highly social nonhuman primates with a prolonged mother-infant relationship and have comparable neuroanatomy (e.g. PFC) and stress physiology to humans. Furthermore, maltreatment occurs in 2-5% (Maestripieri, 1998) of group-living, captive populations, similar to the prevalence rates reported in humans (Brent, Koban, & Ramirez, 2002; Johnson, Kamilaris, Calogero, Gold, & Chousos, 1996; Maestripieri, 1999, 1998; Maestripieri & Carroll, 1998a, b;

Maestripieri, Wallen, & Carroll, 1997a, b; Sanchez, 2006; Sanchez & Pollak, 2009; Sanchez, McCormack, & Maestripieri, 2010; Troisi & D'Amato, 1984), and has been observed in wild macaque groups (e.g., Troisi, D'Amato, Fuccillo, & Scucchi, 1982).

Maternal maltreatment in rhesus monkeys includes both physical abuse and rejection by the mother very early in life, highly co-morbid behaviors distinct from the slaps or rejection behavior exhibited during weaning-related conflicts (Maestripieri, 1998; Sanchez & Pollak, 2009). These behaviors do not occur in competent/nurturing, nonabusive (control) mother-infant relationships. Abuse is operationally defined as violent behavior exhibited by the mother such as dragging, crushing, throwing, stepping or sitting on, or carrying the infant roughly; infant rejection is defined as preventing the infant from making contact, either by holding the infant at a distance with an arm, passively blocking her chest with an arm, or by twisting her torso away from the infant (Maestripieri, 1998; McCormack, Sanchez, Bardi, & Maestripieri, 2006). Maternal maltreatment (both abuse and rejection) triggers behavioral signs of distress (e.g., screams, tantrums) and HPA axis activation in infant macaques (Sanchez, 2006), signs that these are stressful experiences for maltreated infants. As in humans, maternal maltreatment in rhesus monkeys is associated with maladaptive behavioral, socioemotional, and neurobiological consequences (e.g., Grand et al., 2005; Maestripieri et al., 2006; McCormack et al., 2006).

Dimensions of Maternal Care

Recent research highlights the importance of evaluating dimensions of maternal care to assess effects of maltreatment, rather than simply focusing on rates of abuse and rejection (e.g., McCormack et al., personal communication). Attachment theory and attachment research shows that the quality of interaction between mothers and infants has both short and long-term consequences on the developing child, particularly in how they respond to stressful situations

(Ainsworth, Blehar, Waters, & Wall, 1978). In maternal separation trials, securely attached infants are easily comforted by their mothers when they are reunited after a separation, whereas infants who are insecurely attached are unable to be comforted by their mother and remained distressed for much longer than securely attached infants.

Attachment theory also indicates that maltreated infants are more likely than nonabused infants to have insecure attachment with their parent or primary caregiver (Cicchetti, 1989; Cicchetti & Braunwald, 1984; Lyons-Ruth, Connell, Zoll, & Stahl, 1987). The lack of a secure base from which infants can safely explore novel, and potentially stressful, situations (Bowlby, 1969) can have serious clinical implications, including depression, anxiety, heightened suicidality, PTSD, and attention deficit issues (e.g., Harwood 2012). These pathologies directly align with the known consequences of early adverse care (e.g., Beers & DeBellis, 2002). Thus, it is not enough to simply look at rates of abuse and rejection in rhesus monkeys as the sole indicators of maternal maltreatment. Recently, McCormack et al. (personal communication) developed a 22-item scale to better assess specific dimensions of rhesus monkey maternal care that better align with attachment-theory based views of maternal maltreatment. These dimensions include: 1) Sensitivity/Responsivity, 2) Protectiveness, 3) Permissiveness, and 4) Irritability; ratings on this scale can provide a detailed understanding of how specific qualities of maternal behavior affect maltreated infants' behavior, physiology, and cognition.

Gene x Environment Interaction Effects

Historically, allelic variations in the encoding gene for the serotonin transporter (5HTT) promoter region (5-HTTLPR) have been examined as affecting emotionality and the stress response. The gene encoding for the 5-HTT, a molecule responsible for serotonin reuptake, therefore affecting 5HT levels at the synaptic cleft through extracellular clearance, has two

allelic variants, short (*s*) and long (*l*). The *s* allele is associated with lower serotonin reuptake through reduced efficiency of 5-HTT transcription in human and nonhuman primates (e.g., Bennett et al., 2002; Holden, 2003). Traditionally, *l*-variant monkeys tend to be more stress-resilient than their *s*-variant counterparts. There also is some compelling evidence for a functional effect of length polymorphism on cognitive performance in both humans and rhesus monkeys (O'Hara et al., 2007), suggesting that synaptic levels of 5HT affect cognitive function. O'Hara et al. (2007) reported that in older adults (ages 60-100 years) the *s* allele was correlated with poor performance on the Rey Auditory Verbal Learning Test, a measure of delayed recall from verbal memory. Individuals homozygous for the *s* allele (*s/s*) exhibited the poorest verbal memory. More current research, however, has reported that younger adults (ages 18-35 years) carrying the *s* allele performed better on a visual working memory task than individuals with *l*-variants (Anderson, Bell, & Awh, 2012).

Research using rhesus monkeys also has reported effects of 5HTT polymorphisms on cognitive function. For example, on an object discrimination reversal learning (ODRL) task, *s/s* monkeys make significantly more errors before reaching criterion following the first reversal (Izquierdo, Newman, Higley, & Murray, 2007). In the first phase of the ODRL task, two objects were shown to the animals, one baited and one not baited, and the animal was able to displace the object and take the reward if the correct object was chosen. Criterion was met when the animal was accurate 93% of 30 trials one day followed by 80% accuracy the next. The next phase was the first reversal phase in which the original baited or "correct" object became unbaited and incorrect. Izquierdo et al. found that monkeys homozygous for the *s* allele made significantly more errors before reaching criterion on nine reversal trial phases, but not during the initial phase, suggesting that 5HTT genotype has an effect on reversal learning, a task

designed to test for inhibition of behavior. The work of Anderson et al. (2012) and Izquierdo et al. (2007) highlight the importance of addressing potential 5HTT polymorphisms when assessing specific PFC-related cognitive function.

Another potentially valid predictor that researchers are starting to explore is the role of BDNF, or brain derived neurotropic factor. There are a number of developmental changes in neural plasticity that can have lifelong structural and functional effects in PFC pathways of interest (McEwen, 2007). In order to investigate McEwen (2007) and others' (e.g., Smith, Makino, Kvetnansky, & Post, 1995) hypothesis that cognitive deficits associated with early life stress manifest as a result of potentially aberrant changes in neural plasticity in associated areas such as the hippocampus, amygdala, and prefrontal cortex, recent research looking at the role of BDNF in regulating neurobiological effects of early life stress may reveal individual differences in associated plasticity changes (e.g., Roth, Lubin, Funk, & Sweatt, 2009). BDNF is a neurotrophin that is critical for regulating neural integrity and pathway formation. In maternally abused and deprived animals, protein levels and mRNA expression of BDNF are downregulated in animals with exposure to early life stress. Using a rat model of childhood maltreatment with cross-fostering, Roth et al. found that maltreated animals had blunted mRNA expression of BDNF in the PFC, but not the hippocampus (Roth et al., 2009), and that these changes persisted through adulthood. Additionally, these patterns persisted in the next generation of rats, such that maltreated females gave birth to pups who also displayed these patterns. Roth et al. describe an interaction effect of postnatal treatment by the mother, as females who had been maltreated as pups were more likely to display abusive behavior to their own offspring; unlike with first generation animals, however, those who were cross-fostered did not recover down-regulation

patterns of mRNA expression with methylation treatment, indicating the effect of epigenetic factors rather than simply exposure to maternal abuse.

To extend this work, Etzinga and colleagues looked at how polymorphisms of the Val^{66}Met gene, which codes for BDNF, may moderate the effects of the relationship between BDNF and consequences of childhood maltreatment (Etzinga, et al., 2011). Met carriers typically express less secretion of BDNF than Val carriers, and display consequences discussed by previous researchers in regard to down-regulation of BDNF (McEwen, 2007; Smith et al., 1995). In looking at both childhood abuse victims and nonabused individuals, all with a history of Major Depression, Etzinga et al. found that individuals with the Met allele showed reduced serum levels of BDNF when exposed to child abuse. BDNF in Val/Val individuals was not affected by childhood abuse. The work from Roth et al. (2009) indicates the relationship among BDNF, early life stress, and neural plasticity may powerfully affect cognition in maltreated individuals; the work from Etzinga et al. (2011) also supports the importance of the genetic profile, as the Val allele may be the key to having neuroanatomical resiliency in regard to childhood maltreatment (Roth & Sweatt, 2011). While past epigenetic research primarily has focused on effect of 5-HTTLPR polymorphisms on the cognitive consequences associated with early adverse experience, recent research with BDNF highlights the importance of considering alternative gene x environment interactions.

Conclusion

Victims of childhood abuse and neglect often display both internalizing psychiatric disorders, such as clinical depression, generalized anxiety disorder, post-traumatic stress disorder (PTSD), dissociative experiences, and heightened suicidality, as well as greater externalized maladaptive behavior, including episodic aggression, conduct disorder, attention deficit disorder

(ADHD), substance abuse, and greater impulsivity (Hart & Rubia, 2012). Studies using maltreated rhesus monkeys as a translational model for behavioral, emotional, cognitive, and biological consequences of early adverse experience will continue to help researchers better understand how the interface of genetics, neuroendocrine function, and neuromodulatory systems predict consequences of child abuse. The focus of the present review was on the neurobiological substrates of cognitive consequences of early abuse and neglect, particularly on PFC-regulated cognitive processes such as cognitive flexibility, inhibitory control, and memory. As we continue to develop a better understanding of the processes underlying the cognitive consequences of maternal maltreatment using the rhesus monkey model, we also can better identify better forms of prevention, treatment, and protective factors for victims of abuse.

APPENDIX B

LITERATURE REVIEW REFERENCES

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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):

0	**1**	**2**	**3**	**4**	**5**
N/A	**Almost Never**	**Rarely**	**Sometimes**	**Often**	**Always or**
	(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
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

29) How long did the rejections bouts last:

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

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 (> 1 min)

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)?